

## Pharmacognostic Evaluation of *Curcuma longa* L. Rhizome and Standardization of its Formulation by HPLC Using Curcumin as Marker

Abraham A<sup>1\*</sup>, Samuel S<sup>2</sup>, Mathew L<sup>3</sup>

<sup>1</sup>Department of Botany, Mar Athanasius College Kothamangalam

<sup>2</sup>R and D, Kerala Ayurveda Limited Athani

<sup>3</sup>Department of Botany, St Teresa's College Ernakulam

Received: 6<sup>th</sup> Jul, 17; Revised 2<sup>nd</sup> Jan, 18, Accepted: 10<sup>th</sup> Jan, 18; Available Online: 25<sup>th</sup> Jan, 18

### ABSTRACT

Objective: To evaluate *Curcuma longa* rhizome by pharmacognostic and phytochemical analysis and standardize one of its formulations by HPLC. The present work includes macroscopic study, microscopic analysis of cross section and powder of rhizome, fluorescence analysis, qualitative and quantitative phytochemical assay and chromatographic fingerprinting of its polyherbal formulation, Pathyashadangam kwath using curcumin as marker carried out as per standard laboratory procedures. The study helps to provide both diagnostic features for identification and preventing adulteration of *Curcuma longa* L and HPLC chromatogram for standardisation of its formulation. Results: Microscopic analysis revealed the presence of a broad parenchymatous cortex with abundant starch grains in the cross section, spiral vessels, starch grains having hilum towards the narrower end, fibres and cells with oleo resin in powder microscopy. HPLC analysis revealed the presence of three peaks corresponding to curcuminoids in the formulation. Conclusion: Pharmacognostic and phytochemical evaluation can be used for confirming the identity of *Curcuma longa* rhizome and HPLC using curcumin as marker can be used for standardisation of its formulations.

**Keywords:** Curcuma, Pathyashadangam kwath, Curcuminoids, Powder microscopy, Phytochemistry, HPLC.

### INTRODUCTION

*Curcuma longa* L. is a medicinal plant belonging to Zingiberaceae family whose rhizome is widely used in ayurvedic and native medicine against many diseases. At maturity when the leaves of the plant turn yellow the rhizome is carefully dug up with handpicks, cured by boiling and dried. The dried and cured rhizome or haridra is widely used in Ayurveda for treatment of kustha, vrana, tvagroga, prameha and pandu<sup>1</sup>. The rhizome known as Turmeric or yellow spice or spice of life is considered as a great gift of mother-nature because of its curative potential. It has been reported to have antiarthritic, anti-inflammatory, antibacterial, antiallergic and antioxidant effects<sup>2</sup>. Curcumin the active ingredient of the rhizome has been proved to play a key role in the treatment of various pro inflammatory chronic diseases. The enormous therapeutic properties of turmeric rhizome and absence of any significant toxicity has added to its increasing demand for drug manufacturing.

Turmeric has been used in traditional medicine for the treatment of liver and skin diseases, cold and flu and inflammation of joints. It is used singly and in combination with other ingredients to alleviate asthma and cough<sup>3</sup>. The crude extract of turmeric possess selective spasmolytic activity for tracheal and intestinal tissues explaining its traditional use in asthma, cough and diarrhoea<sup>4</sup>. Curcuma is an ingredient of many Ayurveda formulations like

nisatakadi kwath, mahamanjishtadi kwath, vidamkarajanyadi kwath, pathyaamalakadi kwath, pathyapunarnavadi kwath, amritadi kwath and amritarajanyaadi kwath<sup>5</sup>. Curcumin, the active ingredient of turmeric has been proved to improve the overall memory in patients with Alzheimer's disease and hence turmeric has been proved to be beneficial in the prevention and treatment of Alzheimer's disease<sup>6</sup>.

WHO estimates that traditional medicine is used by about 80% of the world population for their primary health care<sup>7</sup>. The first step towards the establishment of identity and purity of drugs can be accomplished by organoleptic, macroscopic and microscopic evaluation and is indispensable before performing other tests<sup>8</sup>. Phytochemical analysis of medicinal plants can pave the way for the development of new drugs by pharmaceutical companies<sup>9</sup>. Quality assurance of herbal drugs by modern techniques and suitable standards is very important. HPLC is a dependable, safe and sensitive method for quality control of drug components. It is a useful tool for standardisation of ayurvedic formulations<sup>10</sup>. Ayurvedic products like dhanyapanchak kwathaurna, guduchyadigana kwathaurna and stanyajanana kashayaurna has been standardised by using HPLC<sup>11</sup>. Pathyashadangam kashayam is a classical ayurvedic polyherbal formulation having *Curcuma longa* rhizome as one of its ingredients. The formulation is used against ear

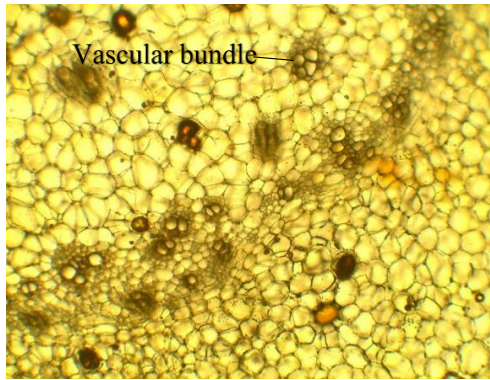


Figure 1: A portion of TS of rhizome.

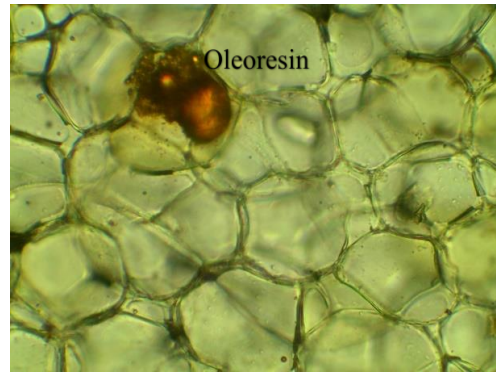


Figure 2: An enlarged portion showing cell with oleoresin.



Figure 3: Fibre and starch grain (Low power view).



Figure 4: Starch grains (High power view).

ache, cluster head ache, migraine, night blindness etc. The present work focuses on pharmacognostic evaluation of *Curcuma longa* rhizome and standardisation of the formulation using curcumin as biomarker.

#### MATERIALS AND METHODS

*Curcuma longa* rhizome was collected from Malippara of Ernakulam district in the month of March. The rhizome was cleaned, scale leaves and roots were removed, washed, cured by boiling and dried in the sun.

The methanol extract of *Curcuma longa* was subjected to phytochemical analysis. Dragendorff's test for alkaloids, Alkaline reagent test for flavonoids, Froth formation test for saponins, Keller Kilani test and Salkowski test for glycosides, Ferric chloride test for tannins, Libermann Burchard test and Salkowski test for steroids was conducted<sup>12</sup>. The total bitter and flavonoids in *Curcuma longa* was quantified by classical method<sup>13</sup>.

**Microscopy:** Transverse section of the rhizome was taken by free hand, mounted in water and observed under the microscope. The dried cured rhizome was powdered and the powder was suspended in water and subjected to microscopic evaluation.

**Fluorescence analysis:** The powder was treated with different reagents and observed under visible, short and long UV lights.

**HPLC analysis:** Pathyashadangam kwath is an ayurvedic formulation referred in Sharnghadhara samhita in which the rhizome of *Curcuma longa* is one of the seven ingredients. The other ingredients are dried fruit pericarps of *Terminalia chebula*, *Terminalia bellirica* and *Phyllanthus emblica*, stem of *Tinospora cordifolia*, stem bark of

*Azadirachta indica* and aerial part of *Andrographis paniculata*. The formulation was standardised by using curcumin as marker. A kashayam was prepared excluding all ingredients of Pathyashadangam kashayam other than *Curcuma longa* and was also subjected to HPLC. The HPLC analysis was carried out using Luna 5u C18 analytical column (250 x 4.6 mm), Shimadzu LC-10 AT vp binary gradient pump and SPD-M10A vp photo diode array detector (PDA) The mobile phase used was A: Acetonitrile (60%) B: 50mM Potassium dihydrogen orthophosphate (pH adjusted to 3.5 using Ortho Phosphoric acid) (40%)<sup>14</sup>. Injection volume was 20 µL and total flow rate was 0.8 ml/min. Isocratic elution was used. Total run time was 12 min, column oven temperature was 250° C and detection wavelength was 450 nm.

#### RESULTS

**Morphology:** It is a group of rhizomes, the central primary rhizome being conical to ovoid in shape with a number of longer secondary rhizomes or fingers attached to it laterally. Both are covered with scale leaves whose remnants are seen as transverse scars and differentiated into nodes and internodes.

**Organoleptic Evaluation:** Fresh rhizomes are light yellow/brown in colour externally and deep orange internally, has characteristic aromatic smell and bitter hot taste.

**Microscopy:** T.S of the rhizome is more or less circular in outline. (Fig 1) The outermost layer is the periderm which consists of 5-6 layers of tangentially elongated cells. This is followed by a broad cortex made of thin walled parenchymatous cells with intercellular spaces. Some cells

Table 1: Powder on fluorescence analysis.

|   | Visible            | Short UV(254 nm) | Long UV (366 nm) |
|---|--------------------|------------------|------------------|
| Powder + HCl                                | Yellow             | Light green      | Black            |
| Powder + Pet Ether                          | Brown              | Green            | Black            |
| Powder + HNO <sub>3</sub>                   | Brown              | Green            | Black            |
| Powder + Acetic acid                        | Fluorescent yellow | Yellow           | Brown            |
| Powder + 50% H <sub>2</sub> SO <sub>4</sub> | Crimson            | Black            | Black            |
| Powder + benzene                            | Light yellow       | Yellowish green  | Coffee brown     |
| Powder + methanol                           | Fluorescent yellow | Yellowish green  | Dark brown       |
| Powder + 50% ethanol                        | Fluorescent yellow | Yellowish green  | Black            |

Table 2: Phytochemical analysis of *Curcuma longa* methanol extract.

| Constituents                        | Methanol Extract |
|-------------------------------------|------------------|
| Flavonoids (Alkaline reagent Test)  | +                |
| Alkaloids (Dragendorff's Test)      | +                |
| Tannins (Ferric Chloride Test)      | -                |
| Sterols (Liebermann- Burchard Test) | +                |
| (Salkowski Test)                    | +                |
| Saponins (Froth formation Test)     | +                |
| Glycosides (Salkowski Test)         | +                |
| (Keller-Kilani Test)                | +                |

contain deposition of orange red substance oleoresin. (Fig 2) Some others contain yellowish oil globules which almost fill the cells. There is a single layered endodermis composed of thin walled rectangular cells. Close to the endodermis many compactly arranged conjoint collateral vascular bundles are present. A number of small bundles are also present scattered inside. Abundant starch grains are found, more in cells lying towards the centre. The starch grains are oblong with hilum towards the narrower end.

#### Powder microscopy

The powder is yellow in colour. Microscopic analysis of the powder revealed the presence of fibres having an average length of  $353.33 \pm 116.71 \mu\text{m}$ , length ranging from  $560 \mu\text{m}$  to  $140 \mu\text{m}$ . Simple starch grains mostly oval and a few round were abundantly found. The average dimensions of the starch grains were found to be  $20.52 \pm 6.73 \mu\text{m}$  by  $14.12 \pm 4.72 \mu\text{m}$ . Spiral vessels having diameter  $42 \mu\text{m}$  to  $56 \mu\text{m}$  were also observed. Cells with oleo resin having an average length of  $107.33 \pm 28.92 \mu\text{m}$  and average breadth of  $74.67 \pm 11.43 \mu\text{m}$  were also found. Preliminary phytochemical analysis of methanol extract of *Curcuma longa* revealed the presence of alkaloids, flavonoids, sterols, glycosides and saponins.

*Curcuma longa* rhizome has been found to contain bitter and flavonoids which may be responsible for its curing potential. The total bitter and flavonoids in the rhizome was quantified (Fig 5).

HPLC analysis of three batches of Pathyashadangam kashayam and Curcuma kashayam was carried out along with curcumin standard. All the three batches of Pathyashadangam kashayam and Curcuma kashayam showed three peaks corresponding to curcuminoids. (Fig 6) The Rt of Curcumin standard was found to be 9.208, that of curcumin in PS 15, PS 23 and PS 25 (three batches of Pathyashadangam kashayam) was found to be 9.285, 9.2

and 9.173 respectively and in Curcuma kashayam was 9.22.

#### DISCUSSION

Microscopic analysis of herbal drugs can ensure their quality and purity and thereby enhance the efficacy and minimise the side effects of herbal medicine. For authentication of commercial samples and detection of adulteration and substitution microscopy can be used as an effective tool<sup>15</sup>. The most accurate identification of ingredients of a polyherbal formulation can be made by taking transverse sections of fresh plant materials and observation and recording of their key anatomical features<sup>16</sup>. *Curcuma longa* rhizome being one of the most widely adulterated spice can be characterised by the oldest, simplest and cheapest method of microscopic analysis<sup>17</sup>. Transverse section of *Curcuma longa* rhizome revealed that the presence of a broad parenchymatous ground tissue containing cells with abundant starch grains and cells with oleo resin and oil globules and conjoint collateral closed vascular bundles are the key diagnostic features of the rhizome. The number and size of starch grains is an important feature for identification of Curcuma species. The curing percentage, oleoresin, oil and percentage of curcumin can be used as quality parameters for the evaluation of *Curcuma longa* rhizome<sup>18</sup>. The powder microscopic analysis revealed that starch grains having hilum towards the narrow end and cells with oleoresin can be used for confirming the identity of the powder of *Curcuma longa* rhizome.

Flavonoids are water soluble compounds which usually occur in plants as mixtures and show intense absorption bands in UV and visible regions<sup>19</sup>. *Curcuma longa* rhizome contains many types of flavonoids and the isolated flavonoids have reducing power and strong antioxidant activity as is indicated by its capacity to scavenge hydrogen peroxide radicals<sup>20</sup>. Flavonoids from food sources have versatile health benefits because of their antioxidant, free radical scavenging, anticancer and other activities and hence it is important to evaluate flavonoid sources in foods<sup>21</sup>. Quantification of the total flavonoid content of *Curcuma longa* rhizome is important as it is a commonly used spice and a widely used ingredient of herbal and ayurvedic formulations.

Chromatographic techniques can be used for authentication of turmeric products and curcumin, demethoxy curcumin and bisdemethoxy curcumin can be used as marker compounds for the quality control of *Curcuma longa* rhizomes, powders and extracts<sup>22</sup>.

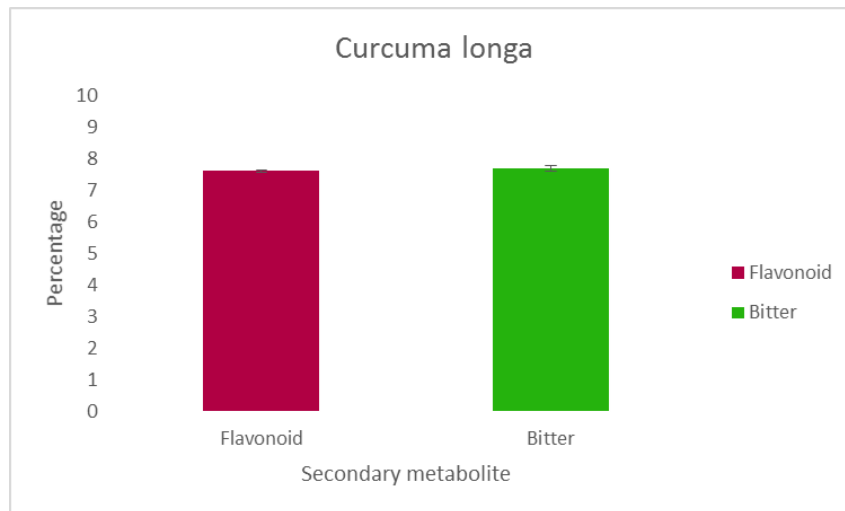
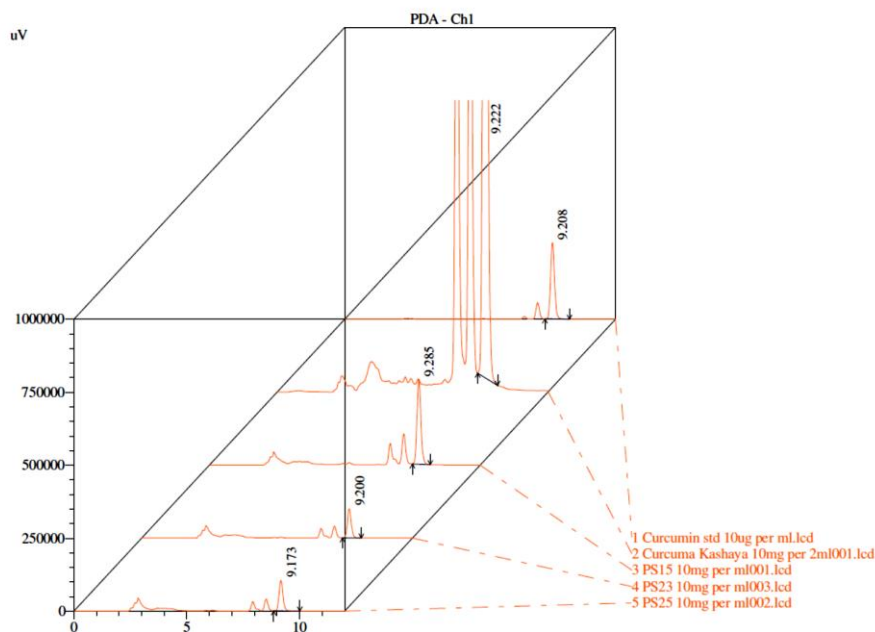
Figure 5: Total bitter and flavonoids in *Curcuma longa* rhizome.

Figure 6: HPLC fingerprint of standard curcumin, Curcuma kashayam and three batches of Pathyashadangam kashayam PS 15, PS 23 and PS 25.

Kalyanavaleha a polyherbal formulation used in aphasia has been standardised by HPTLC using Curcumin as biomarker<sup>23</sup>. HPLC analysis of kashayam was carried out to confirm the presence of curcumin so that it can be used as marker for standardizing such formulations. The observation of a peak corresponding to Curcumin in standard, three batches of Pathyashadangam kashayam and Curcuma kashayam at similar Rt shows that HPLC by using curcumin as marker can be used for the standardisation of polyherbal formulation, Pathyashadangam kashayam and other formulations having *Curcuma longa* rhizome as an ingredient.

## CONCLUSION

The rhizome of *Curcuma longa* was subjected to organoleptic, morphological, anatomical, phytochemical and chromatographic analysis. Organoleptic evaluation of the rhizome revealed that it has yellow colour, aromatic

odour and bitter hot taste. Anatomical evaluation revealed that a periderm made of tangentially elongated cells, broad cortex made of parenchymatous cells with intercellular spaces, presence of starch grains, oleo resins and volatile oil in cells of the cortex and collateral closed vascular bundles are diagnostic characters of the rhizome. From the powder microscopic analysis of the rhizome it was concluded that the presence of fibres, starch grains having hilum towards the narrow end and cells with oleo resin are key identifying features. Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, sterols, glycosides and saponins in the methanol extract of the rhizome. HPLC analysis of curcumin, Curcuma kashayam and Pathyashadangam kashayam, an ayurvedic formulation containing *Curcuma longa* as an ingredient confirmed that curcumin can be used as a marker for standardization of formulations containing turmeric as is evidenced from the similarity in

Rt of peaks corresponding to Curcumin in the standard and kwath.

## REFERENCE

1. Anonymous, *The Ayurvedic Pharmacopoeia of India*, Part I, Volume I, First Edition, (Government of India, Ministry of Health and Family Welfare Department of Ayush), (1999), Page 45.
2. Rajpal V, *Standardization of botanicals* (Testing and extraction methods of medicinal herbs), Volume 1, Eastern publishers, G 59, Greater Kailash – 2, New Delhi, (2002), 131.
3. Shahnaz Sultana, Andleeb Khan, Mohammed M Safhi and Hassan A. Alhazmi. Cough Suppressant Herbal Drugs: A Review. *International Journal of Pharmaceutical Science Invention*. Volume 5, Issue 5. (2016), 15-28.
4. Anwarul Hassan Gilani T, Abdul Jabbar Shah, Muhammad Nabeel Ghayur, Kashif Majeed. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. *Life Sciences*, 76, (2005), 3089 – 3105.
5. Krishnavaidyan K V A and Gopalapilla S A, *Sahasrayogam*, Vidyarambhan publishers, Alappuzha, 1992.
6. Shrikant Mishra, Kalpana Palanivelu. The effect of curcumin (turmeric) on Alzheimer's disease: An overview. *Annals of Indian academy of neurology*, Volume 11, Issue 1, (2008), 13-19.
7. World Health Organization: *Quality control methods for medicinal plant materials*, WHO Library, 1998, 110-115.
8. Sandhya B, Thomas S, Isabel W and Shenbagarathai R. Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (reserved forest), Tamilnadu, India. - A pilot study. *Afr. J. Trad. CAM*, 3 (1), (2006), 101 – 114.
9. Abdul Wadood, Mehreen Ghufuran, Syed Babar Jamal, Muhammad Naeem, Ajmal Khan, Rukhsana Ghaffar and Asnad. Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. *Biochem Anal Biochem*, (2013), 2-4.
10. Singh D P, Govindarajan R and Rawat A K S. High-performance Liquid Chromatography as a Tool for the Chemical Standardisation of Triphala—an Ayurvedic Formulation. *Phytochem. Anal.* 19, (2008), 164–168
11. Santosh M K, Shaila D and Sanjeeva Rao I. Standardisation Study of Kwatha Curnas. *E-Journal of Chemistry*, Vol. 1, No. 5, (2004), 251-255.
12. Kokate C K, Purohit A P & Gokhale S B, *Pharmacognosy*. Forty Eighth Edition. (Nirali Prakashan), A21-26.
13. Rajpal V, *Standardization of botanicals* (Testing and extraction methods of medicinal herbs), Volume 1, Eastern publishers, G 59, Greater Kailash – 2, New Delhi, (2002), 124-215.
14. Boominathan U and Sivakumaar P K, A liquid chromatography method for the determination of curcumin in PGPR inoculated *Curcuma longa*. L plant. *IJPSR*, Vol. 3(11), (2012), 4438-4441.
15. Sayyada Khatoon. Macro-Microscopy & Planar Chromatography-Important Tools for Quality Control and Identification of Adulterants/Substitutes of Unani Drugs. *International Journal of Advances in Pharmacy Medicine and Bioallied Sciences*, Volume 3, Issue 1, (2015), 58-64.
16. Hetal Janani, Jayanta Kumar Maji and Ashish Verma. Standardization of anticonvulsant polyherbal formulation by pharmacognostical, DNA fingerprinting and high-performance thin layer chromatography combined with chemometrics. *Int J Curr Pharm Res*, Vol 9, Issue 1, 50-58.
17. Bouzabata Amel. Microscopic analysis of *Curcuma longa* L. using multivariate test. *IJP*, Vol. 2, Issue 4, (2015), 173-177.
18. Ratnambal M J. Evaluation of turmeric accessions for quality. *Qual Plant Plant Foods Hum Nutr* 36, (1986), 243-252.
19. Harborne J B, *Phytochemical methods*, A guide to modern techniques of plant analysis, Springer (India)private limited, New Delhi, (2005).60.
20. Abdul Monaim Hamad Majeed Al-Samarrai, Khalaf Faris Al-Samarrai and Mithaq Nazhan Mahmood. Isolating and identifying flavonoids of the *Curcuma* plant sp. (*Curcuma longa*) and studying their effect as antioxidant in vivo and in vitro. *Asian Journal of Science and Technology* Vol.06, Issue, 11, (2015), 1955-1959.
21. Yao L H, Jiang Y M, Shi J, Tomás-Barberán F A, Datta N, Singanusong R, Chen S S. Flavonoids in food and their health benefits. *Plant Foods Hum Nutr*, 59(3), (2004), 113-22.
22. Shiyu Li, Wei Yuan, Guangrui Deng, Ping Wang, Peiyong Yang and Bharat Aggarwal B. Chemical Composition and Product Quality Control of Turmeric (*Curcuma longa* L.) *Pharmaceutical Crops*, 2, (2011), 28-54.
23. Sayyada Khatoon, Harsh Singh, Anshu Rathi, Sanjeev Kumar Ojha, AKS Rawat. Standardisation of an ayurvedic formulation kalyanavaleha and estimation of curcumin using HPTLC. *Indian journal of traditional Knowledge*, Vol. 13(3), (2014), 535-542.