**Journal of Pharmaceutical and Scientific Innovation**
**www.jpsionline.com**
**Research Article**

# A PHARMACOGNOSTICAL STANDARDISATION STUDY ON *TOONA CILIATA* M. ROEM., BARK

Mann Avninder Singh[1], Sandhu Bharpur Singh[2], Dwivedi Gopal Krishna[3], Kaushik Rajan[*4]
[1]Head & Professor, Department of Pharmacognosy, B.I.S. College of Pharmacy, Gagra, Moga (Punjab) –142001, India
[2]Assistant Professor, Department of Pharmacognosy, B.I.S. College of Pharmacy, Gagra, Moga (Punjab) – 142001, India
[3]Professor, Department of Biotechnology, Institute of Biotechnology, G.B. Pant University of Agriculture & Technology, Patwadangar, Nainital (Uttarakhand) –263128, India.
[*4]M.Pharm Research Scholar, Pharmacognosy Research Lab, Department of Pharmacognosy, B.I.S. College of Pharmacy, Gagra, Moga (Punjab)- 142001, India.
*Rajan Kaushik, M.Pharm Research Scholar, Pharmacognosy Research Lab, Department of Pharmacognosy, B.I.S. College of Pharmacy, Gagra, Moga (Punjab) – 142001, India Email: rajankaushik96@gmail.com, raj_pharmachem@yahoo.in

Received on:23/04/2012 Revised on:21/05/2012 Accepted on: 12/06/12

**ABSTRACT**
*Toona ciliata* M. Roem formerly called as *Cedrella toona* Roxb, known as Tooni in Sanskrit and Red cedar in English is a very important medicinal plant of renowned Meliaceae family. Tooni is used in our traditional system of medicine for the cure and prevention of various ailments viz. Antileprotic, bitter tonic and as anthelimintic. In the present study an attempt was made to study the pharmacognostical features of fresh and dried bark of *Toona ciliata*. Organoleptic (Colour, odour & taste), Macroscopic (Size, shape, texture & fracture) and Microscopic (Powder microscopic characteristics) evaluations were performed to establish the qualitative and diagnostic features. The various physiochemical parameters (Loss on drying, foreign matter, extractive values, ash values, pH, percent crude fibres) were also determined for the effective standardisation of the medicinal plant material. Beside all above mentioned conventional methods of standardisation, the powdered bark was also subjected to powder drug analysis with different chemical reagent and for fluorescence drug analysis on exposure to different wavelengths of ultraviolet light. The various determinations and analysis which were carried out in the present study will certainly help for first line identification and quality control of plant material in its crude form. Further, the authentic material can be subjected to the phytochemical investigations, isolation of phytochemicals and many more for the validation of vast traditional therapeutic potential of the plant material in terms of its in-vivo pharmacological screening.
**Keywords:** *Toona ciliata*, Red Cedar, Pharmacognostic, Standardisation, Microscopy, UV-Visible, Fluorescence.

## INTRODUCTION

Fossil records date human use of plants as medicines at least to the Middle Palaeolithic age some 60,000 years ago[1]. The human's existence on this earth has been made possible of the vital role played by plant kingdom. Nature has provided many things for humankind over the years, including the tools for the first attempts at therapeutic intervention[2]. The World Health Organization (W.H.O.) estimates that 80% of the people living in developing countries almost exclusively use traditional medicine. Such medicines, derived directly or indirectly from plants, constitute 25% of the pharmaceutical arsenal. *Toona ciliata* M. Roem., commonly known as *Red cedar* throughout the world, is the member of renowned *Meliaceae* family[3], Figure 1.0 & 2.0. The plant is official in Ayurvedic Pharmacopoeia of India and mentioned as a plant with medicinal properties like tonic, astringent, anthelimintic, antiperiodic and antiulcer in several traditional ayurveda literature. The stem bark of the tree is used as antiulcer, antileprotic and had been used traditionally to heal wounds[3, 4, 5]. The leaves have hypoglycaemic, spasmolytic and anti-protozoal activity. Its flowers decoction also act as powerful emenagogue and useful in menstrual disorders [3]. Gum obtained from the bark is useful in fever[6]. Garasia tribe of Rajasthan applies the leaves as bandage tied on the stomach to reduce swelling occurs during pregnancy, where they called it locally as 'Bhurla'[7]. The inhabitants of Abbotabad district of Pakistan uses the dried leaf powder along with table salt and water orally for treating diabetes, skin allergy, wounds and as blood purifier, where they pronounced the plant locally as 'Nem'[8]. The plant is a rich source of several phytoconstituents viz. alkaloids like furoquinoline alkaloid (skimmianine and 2-hydroxy-4-methoxy cinnamaldehyde) coumarins glycosides (isopimpinellin and siderin), flavanoids (quercetin) from dyestuff, the phytosterols (sitosterol and stigmasterol), tannins, resins, colouring matter (nyctanthin, triterpenoids), essential and fatty acids [9,10,11].

## MATERIALS AND METHODS

### Collection and Authentication

The plant materials i.e. stem bark pieces were collected from the matured tree of the *Toona ciliata* from the rocky hill slopes of Village- Patwadangar, District: Nainital of Uttarakhand, India in the month of July-2011; Figure 1.0. The bark was identified and authenticated vide Ref. No.- NISCAIR/RHMD/Consult/-2011-2012/1997/05 by Dr. H.B. Singh, Chief Scientist & Head, Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources, a constituent establishment of Council of Scientific and Industrial Research, New Delhi. The collected stem bark pieces were washed with distilled water, placed on bloating paper and left for shade drying at room temperature (23±1°C). After 25 days of complete shade drying, the bark pieces were finally powdered using electrical blendor (Waring Corporation, USA) and passed separately through # 40 and #80 sieves to get powders of different particle size. The powders thus prepared were stored in airtight plastic containers till used for further study.

### Chemicals and Reagents

All the analytical grade reagents and chemicals of HIMEDIA, Mumbai, India, were employed during the tenure of research work.

### Organoleptic evaluation

The pieces of freshly peeled stem bark as well as shade dried bark were evaluated for sensory characteristics viz. colour

and condition under visible and artificial light, odour and for taste[12,13,14].

**Macroscopic evaluation**

The pieces of freshly peeled stem bark were spread over a clean white paper sheet, investigated for size, shape, texture and fracture repeatedly using scale (wherever required), magnifying hand lens (6X) and under simple microscope (10 X) (Scientech, India). Similarly after 25 days of complete shade drying, shade dried bark pieces were subjected to macroscopic evaluation[12, 13,14,15,16].

**Microscopic evaluation**

A small quantity of powder was cleared using chloral hydrate solution with little heating. The cleared powder was subjected to staining with the microscopy chemical reagents to identify the characteristic microscopic features[12,13,14].The properly stained slides were observed under 10X magnification on a digital Trinocular Research Microscope (Nikon Eclipse-80*i*, Japan) and representative microscopical features were documented using photomicrography facility provided with microscope.

**Physiochemical evaluation**

The physiochemical characters viz. Foreign matter, loss on drying, extractive values (Water ,alcohol and ether soluble), ash values (Total and acid insoluble), percentage crude Fibres, pH (1% solution) were determined according to official methods[12,13,14,15,16].

**Fluorescence and Powder drug analysis**

A small quantity of the finally powdered stem bark was spread onto a grease free microscopic slide and placed in a UV-visible light cabinet (Popular India).The visible lamp was switched on and observed for responding colour. Thereafter the slide was observed under UV short (254 nm) and UV long (365 nm) light lamps simultaneously. In the second experiment an equal amount of powdered bark was spread over the microscopic slides and to the each slide a different chemical reagent was added and mixed by gentle tilting. After 2 minutes, the slides prepared were placed into a UV-Visible light cabinet and viewed in day, UV short (254) and UV long (365) radiations[17]. The colours observed after application of different chemical reagents in different radiations were then recorded.

**RESULTS & DISCUSSION**

In the present study, fresh as well as shade dried bark of *Toona ciliata* was subjected to pharmacognostical standardisation using conventional and modern method of herbal drug standardisation. Standardisation of herbal raw material is very important today due to the following reasons viz. Biochemical variation in the drug, deterioration due to treatment & storage and substitution & adulteration[18]. Initially standardisation was based on comparison to with a reference sample but now a day for such the purpose, arrays of analytical methods are available for confirming the identity, determination of quality and purity and detection of deterioration or adulteration. With the modernisation of field of chemical analysis, it is now possible to estimate the total chemical constituents and physical parameters from the crude drugs, which helps in both qualitative and quantitative type standardisation. Organoleptic evaluation which is also called as sensory evaluation is based on identification of drug in visible as well as in artificial light, where a crude drug is judged for identity, quality and purity on the basis of sensory characters viz. Colour, condition, odour and taste. According to World Health Organisation (W.H.O.) medicinal plant materials are categorized according to sensory, macroscopic and microscopic characteristics. Therefore, an examination to determine these characteristics is the first step towards establishing the identity and the degree of purity. The results of organoleptic evaluation are given in table 1.0, shows that fresh bark is greyish green externally and creamish brown internally. Fresh bark is moist in nature and has pleasant aromatic and characteristic odour of *Cedrella*. Fresh bark has bitter taste with significant astringency which indicates the presence of tannins and other bitter chemical constituents. On shade drying, significant changes have been noted in bark, the colour of the dried bark gets change to greyish black externally and brown internally which make it different in colour from fresh bark. The odour gets change to mild aromatic which indicate the loss of volatile components which would probably be responsible for strong aromatic odour, when the bark was fresh. Similarly the taste gets change to slight bitter with mild astringency indicates the changes in phytochemicals after shade drying. The results of macroscopic evaluation are given in table 2.0. Macroscopic evaluation of the freshly peeled bark shows that fresh bark occurs in flat pieces of varying dimensions. Transversely broken surface of bark shows numerous fibers indicate that the bark has fibrous and short fracture. The outer surface of fresh bark produces the sensation of rough surface which indicates the presence of rough texture. Similarly macroscopic evaluation of shade dried bark shows numerous distinguishing characteristics viz. on shade drying the bark pieces get transformed form flat pieces to channelled and/or quilled shape pieces of varying dimensions. Fracture and texture were noted similar to fresh bark but bark get hardened with contraction on shade drying. The outer surface of dried bark shows characteristics shallow reticulate cracks and inner surface shows the presence of numerous longitudinal striations, which are important characteristic macroscopic features for identifying the dried bark material. Results of qualitative microscopy of powder bark material shows the presence of pointed lignified fibres; Figure No. 6.0, fragments of polygonal cork cells; Figure No. 7.0, scattered stone cells, few in groups; Figure No.8.0 and multi component starch grain; Figure No. 9.0, which all are characteristic diagnostic features to identify the powdered material of the plant species bark material on the basis of microscopy. The results of physical evaluation are tabulated in table 4.0, shows none presence of foreign matter or contamination by insects, maximum percentage extractive yield with alcohol indicates that the bark contains polar phytochemicals, which suggest that the alcohol can be used as a maximum extractive yielding solvent in further studies. A minimum percentage extractive yield with ether shows that plant bark material contains a very small amount of fatty material. A high value of percentage crude fibres indicates that the bark is hard and has a good constructional pattern at cellular level. A small value of loss on drying infers that dried powdered bark contains moisture in limit (less than 10) which is good information on its stability. The pH value 6.4 of 1% w/v aqueous solution of powdered bark indicates that bark contains water soluble acidic compounds; Figure 5.0. In addition to conventional methods, some modern methods were also employed for standardisation of bark material like fluorescence cum powder drug analysis[17]. From many of

researches it has been successfully proved that, modern methods like powder drug analysis and fluorescence drug analysis are very useful in standardisation of plant material. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range of radiation in daylight. The ultra violet light produces fluorescence in many natural drugs (e.g. berberis, cinchona, emetine etc), which do not show fluorescence in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is also an important parameter of pharmacognostical evaluation[15]. The results of fluorescence cum powder drug analysis are given in table 5.0., shows the different types of response exhibited by the powder bark alone and after its treatment with chemical reagents on being exposing to different wavelengths of UV-Visible radiation. Conclusively all the studies carried out in the present study will serve as a reference standard to this morphologically variable species of Genus *Toona*.

## CONCLUSION

The standardisation of raw material is of utmost requirement before subjecting the material to biological screening. The World Health Organization (W.H.O.) encourages, recommends and promotes traditional /herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The World Health Organization (W.H.O.) assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. In the present study, the bark material from the *Toona ciliata* was subjected to various conventional and modern method of medicinal plant material standardisation. The presence of pointed lignified fibres, polygonal cork cells, isodiametric stone cells and multi component starch grains are important characters for microscopic identification. From the pharmacognosy point of view maximum and minimum extractive yields with ethanol & petroleum ether respectively, presence of significant amount of fibres, none presence of the foreign organic matter are all the important parameters to judge its quality and purity. Last but not least, the various evaluations and analysis which were carried out in the present study will certainly help in first line identification and quality assurance for this plant species bark material.

## ACKNOWLEDGEMENT

Corresponding author is highly thankful to Prof. R.S. Chauhan, Campus Director, Institute of Biotechnology, Patwadangar (Nainital), Uttarakhand, India, an outstation research centre of G.B. Pant University of Agriculture and Technology, Pantnagar (Uddham Singh Nagar), Uttarakhand, India for providing premium research facilities during the tenure of project work.

## REFERENCES

1. Solecki R. Shanidar IV- a Neanderthal flower burial in northern Iraq. Science 1975; 190: 880–881.
2. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bullet WHO 1985; 63: 965-81.
3. Joshi SG. Medicinal Plants. 1st ed., New Delhi: Oxford & IBH Publishers; 2000: 264
4. Gangwar KK, Deepali, Gangwar RS. Ethnomedicinal plant diversity in Kumaun Himalaya of Uttarakhand, India. Nat & Sci 2010; 8(5): 66-78.
5. Sashtri BND, editor. Bhavaprakashnighantu. 6th ed., Delhi: Motilal Banarasidas Publication; 1966: 351.
6. Narayanan Ratheesh MK *et al.* Ethnobotanically important trees and their uses by Kattunaikka tribe in Wayanad Wildlife Sanctuary, Kerala, Indian J Med Plant Res 2011; 5(4): 604-612.
7. Meena KL, Yadav BL. Some ethomedicinal plants used by the Garasia tribe of district Sirohi, Rajasthan. Indian J Trad Know 2011; 10(2): 354-357.
8. Abbasi AM, Khan MA, Ahmad M, Zafar M. Herbal medicines used to cure various ailments by the inhabitants of Abbottabad district, North West Frontier Province, Pakistan. Indian J Trad Med 2010; 9(1): 175-183.
9. Negi JS, Bisht VK, Bhandari AK, Bharti MK, Sundriyal RC. Chemical and Pharmacological aspects of Toona (Meliaceae). Res J Phytochem 2011; 5(1): 14-21.
10. Malairajan P, Gopalakrishnan S, Narasimhan S, Veni KJK, Kavimani S. Anti-ulcer activity of crude alcoholic extract of Toona ciliata Roemer (heartwood). J Ethnopharm 2007; 110(2): 348-351.
11. Chowdhury R, Ahmed A, Rahman MA. 7-Hydroxy-4-methoxy-5-methylcoumarin: A further 5-Methylcoumarin from Toona ciliata (Meliaceae). Nat Prod Sci 2004, 10(3): 99-100.
12. Mukharjee PK. Quality control of herbal drugs- An approach to evaluation of botanicals. 1st ed., New Delhi: Business Horizons Pharmaceuticals Publications; 2002
13. Khandelwal KR. Practical Pharmacognosy-Techniques and Experiments. 19th ed., Pune: Nirali Prakashan; 2008: 157-160
14. Kokate CK, Purohit AP, Gokhale SB. Practical Pharmacognosy. 13th ed., New Delhi: Vallabh Prakashan; 1996: 123-125
15. Bhattacharya S, Zaman MK. Pharmacognostical evaluation of Zanthoxylum nitidum Bark. Int J Pharm Tech Res 2009; 1(2): 292-298.
16. Government of India. Ministry of health and family welfare. Indian Pharmacopoeia Vol. II. The Controller of Publication, New Delhi; 1996: A-53-A-54
17. Kokoski J, Kokoski R, Salma FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. J Amer Pharm Assoc 1958; 47: 715-717.
18. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. 44th ed., New Delhi: Nirali Prakashan; 1994: 6.3.

**Table 1.0: Results of organoleptic evaluation**

| S.No. | Parameter | Fresh bark | Shade dried bark |
|---|---|---|---|
| 1. | Colour | | |
| | Inner Surface | Greyish green | Greyish black |
| | Outer Surface | Creamish brown | Brown |
| 2. | Condition | Moist | Dry |
| 3. | Odour | Aromatic characteristic | Mild Aromatic characteristics |
| 4. | Taste | Bitter with astringency | Slightly bitter with astringency |

**Table 2.0: Results of macroscopic evaluation**

| S.No. | Parameter | Fresh bark | Shade dried bark |
|---|---|---|---|
| 1. | Texture | Rough | Rough |
| 2. | Fracture | Short & Fibrous | Short & Fibrous |
| 3. | Size | Variable | Variable |
| 4. | Shape | Flat pieces | Channelled to quilled |

**Table 3.0: Micro chemical tests performed on powder bark of *Toona ciliata***

| S.No. | Reagent | Observation | Diagnosis |
|---|---|---|---|
| 1. | Phloroglucinol: Con HCl | Pink | Lignified fibres, cork cell, stone cell |
| 2. | Dilute Iodine | Blue-Black | Starch Grains |
| 3. | Hydrochloric Acid | Effervescence/ Soluble crystal | Calcium Oxalate |

**Table 4.0: Results of physiochemical evaluation**

| S.No. | Parameter | Obtained value |
|---|---|---|
| 1. | **Extractive values** | |
| | (a) Water soluble extractive | 15.6 % w/w |
| | (b) Alcohol soluble extractive | 16 % w/w |
| | (c) Ether soluble extractive | 1% w/w |
| 2. | **Ash values** | |
| | (a) Total ash | 8.2% w/w |
| | (b) Acid insoluble ash | 2.3% w/w |
| 3. | **Foreign organic matter** | Nil |
| 4. | **Loss on drying** | 8% |
| 5. | **Percentage crude fiber** | 42.85% |
| 6. | **pH value (1% solution)** | 6.4 |

**Table 5.0: Results of fluorescence and powder drug analysis**

| Material | Visible light | UV-short (254nm) | UV-long (365nm) |
|---|---|---|---|
| *PBM alone | Light brown | Greyish Green | Black |
| PBM + Acetic acid | Brown | Dark brown | Black |
| PBM + 5% $FeCl_3$ in ethanol | Greenish black | Dark greenish black | Black |
| PBM + Petroleum ether | Brown | Dark brown | Black |
| PBM + Methanol | Brown | Dark brown | Black |
| PBM + Saturated Picric acid | Yellow | Dark brown | Black |
| PBM + 1% Picric acid | Yellow | Dark brown | Black |
| PBM + 25% Liquid ammonia | Dark brown | Intense dark brown | Black |
| PBM + 1M NaOH | Reddish brown | Dark brown | Black |
| PBM + Con. HCl | Reddish brown | Green | Black |
| PBM + 1M $H_2SO_4$ | Brown | Emerald green | Black |
| PBM + 1M HCl | Brown | Green | Black |
| PBM + Ninhydrin solution | Brown | Dark brown | Black |

*PBM – Powdered bark material

**Figure 1.0: The matured green *Toona* tree**

**Figure 2.0: The stem of matured *Toona* tree**

**Figure 3.0: The freshly collected bark pieces**

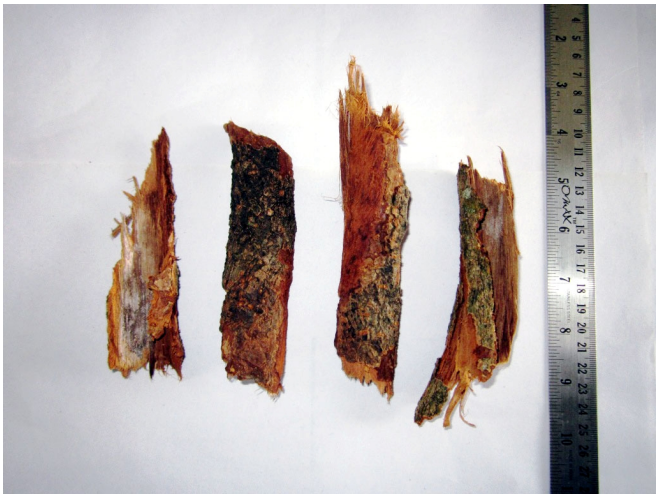

**Figure 4.0: Measurement study on shade dried bark pieces of *Toona***


**Image 5.0: Measurement of pH of 1% w/v aqueous solution of powdered bark by pH meter (Standard glass electrode, pHep-HI98107, Hanna Instruments, Mauritius)**

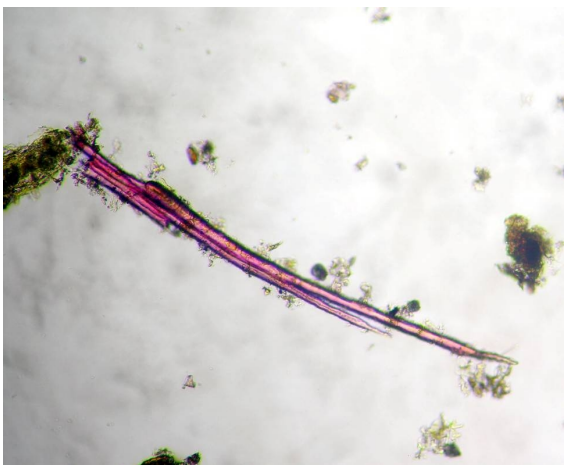

**Figure 6.0: Pointed lignified fibres**

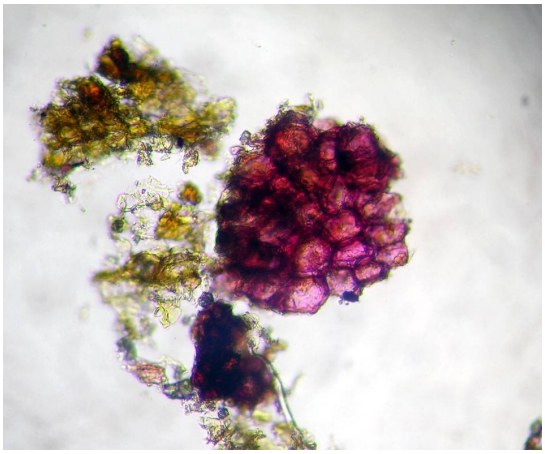

**Figure 7.0: Fragments of polygonal cork cells**

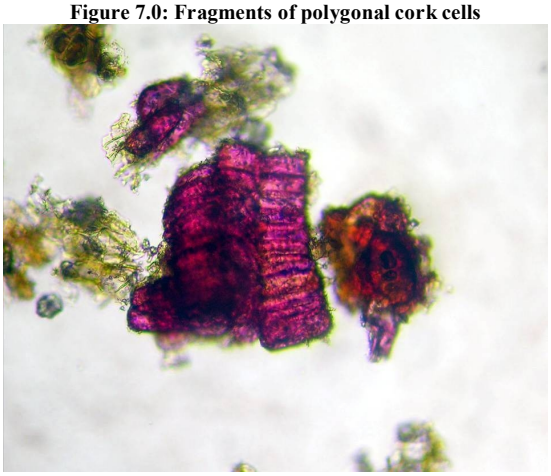

**Figure 8.0: Isodiametric stone cells in group**

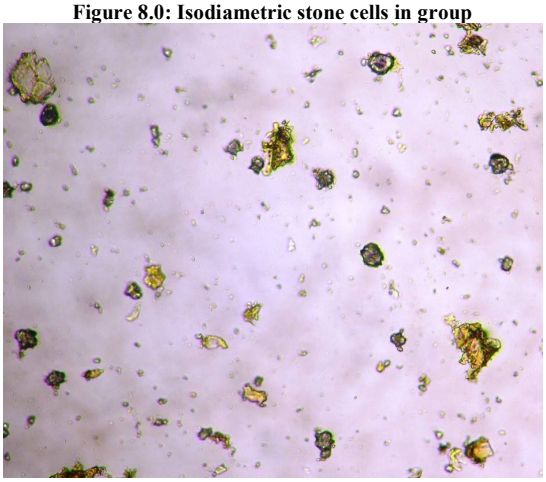

**Figure 9.0: Multicomponent starch grains**