WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 376-382. Conference Article ISSN 2277– 7105

STANDARDIZATION AND AUTHENTICATION OF SEEDS OF AJMODA (*APIUM GRAVEOLENS*): THE DRUG ASSESSED FOR CHEMO PREVENTIVE ACTIVITY

Sagarika Parida*[1], Kedar Kumar Rout[2], Gyanranjan Mahalik[3], Nabin Kumar Dhal[4], Kunja Bihari Satpathy[5]

[1]Asst. Professor, Dept. of Botany, Centurion University of Technology and Management, Bhubaneswar.

[2]Reseach Associate, OUAT, Bhubaneswar.

[3]Lecturer, Dept. of Botany, Centurion University of Technology and Management, Bhubaneswar.

[4]Scientist, Institute of Minerals and Materials Technology, CSIR.

[5]Emeritus Professor, Dept. of Botany, Centurion University of Technology and Management, Bhubaneswar.

Article Received on
19 March 2018,

Revised on 09 April 2018,
Accepted on 29 April 2018,

DOI: 10.20959/wjpr20189-12116

***Corresponding Author**
Sagarika Parida
Asst. Professor, Dept. of Botany, Centurion University of Technology and Management, Bhubaneswar.

ABSTRACT

India is endowed with a rich wealth of medicinal plants. A number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Siddha and Unani, popularly known as Indian System of Medicine (ISM) which have been widely practiced due to its efficacy and believed to be free off side effects. Literature data revealed that search for anticancer drugs from plant sources started in earliest in the 1950s and *Apium graveolens* seeds have been assessed for chemopreventive activity. Chemopreveventive includes use of synthetic or natural agents alone or in combination to block the development of cancer in human beings leading to cancer prevention. It is also revealed that *A. graveolens* have many pharmacological properties like anticonvalscent, tranquilizing, hypolipidemic, antifungal activities, anticancer and many more. The seeds are also used in bronchitis, asthma, spleen and liver disorders. This has increased the demand for plant raw materials with the result that a large number of traders connected with big industrial houses have started exploiting the plants indiscriminately. The raw materials of herbal drugs, which are mostly, collected wild and the

supply line is adversely affected, leading to adulteration and substitution for genuine drugs. As the medicinal plants are collected by untrained collectors basing on the Sanskrit names or local names, there is a chance of misidentification out of ignorance due to similar vernacular name. The present study was conducted with an aim of developing standard marker for proper identification of raw materials. In this investigation, the parameters adopted for identification of seeds of *A. graveolens* include physico-chemical values, fluorescence analysis and powder microscopy to develop their chemical parameters and diagnostic microscopic characters.

KEYWORDS: 'Ajmoda', pharmacognosy, *Apium,*

INTRODUCTION

Medicinal plants are a rich source of pharmacologically active compounds and are used traditionally by various cultural groups to treat various diseases including cancer. These plants were identified as medicines through human experiences. Many plant based drugs containing vincristine, vinblastin, camptothesin and taxol are used as chemotherapeutic agents in recent days. All parts of the plants of *Apium graveolens* L. especially seeds are used to treat and prevent diverse types of cancers. Apoptosis which is the body's own mechanisms to remove unwanted cells including cancer cell motivates many researchers to screen the phyto-chemicals of medicinal plants and their parts for anti-cancer activity. Many medicinal plants extract and photochemical are known to induce apoptosis.[1] Literature data revealed that *Apium graveolens* seeds have been used as to treat and prevent various types of cancer because of its antioxidant properties.[2]

Apium graveolens L. is called 'ajmoda' in Hindi and 'celery' in English grows wild in the foothills of North Western Himalayas and outlying hills of Punjab, Himachal Pradesh and Utter Pradesh (Anonymous, Wealth of India, 1985). It is largely cultivated in Amritsar and adjoining areas of Punjab, Haryana and some localities of Utter Pradesh for its seeds which are exported as condiment. Leaves are used as vegetable. Entire plant is endowed with many medicinal properties.[3,4] The fruits is schizocarpic, sub-orbicular to ellipsoid, 1-2 mm in diameter, aromatic and slightly bitter.[5-7] The seeds are stimulative and used as cardiac tonic. Their decoction is a popular house hold remedy of arthritis, rheumatism and gout by flushing away the uric acid crystals that build up around the joints along with anti-inflammatory properties and thereby reducing joint pain. The seed also has antiseptic and diuretic properties and is useful in treating fluid retention. It is also used in bronchitis, asthma, spleen and liver

disorders. It has shown antifungal, anticonvalscent, tranquilizing and nerve stimulant activities suggesting their therapeutic utility in various psychiatric, epilepsy-like diseases. The celery seeds contains cancer fighting substances including apigenin, apiuman, luteoline, chrysoeriol, coumarin and several polycetelenes and polyphones and also contain perillyl alcohol which has been found to have anticancer activity.[8,9] Apigenin is found as one of the major active ingredient that inhibited the growth of many human cancer cell lines like cervical carcinoma cells, breast cancer cells and leukomia through apoptosis activity.[10,11,12] The apigenin anticancer effect has been found to be mediated through induction of p53 expression, which causes cell cycle arrest and apoptosis.[13] Tannins are also extracted from *A. graveolens* seeds and they are considered to have cancer preventive properties.[14] It is reported that seeds of *A. graveolens* contain high levels of vitamins A, B and C which are antioxidants and are useful in reducing the oxidative stress caused by toxic agents.[15]

As the medicinal plants are collected by untrained collectors basing on the Sanskrit names or local names, there is a chance of misidentification out of ignorance due to similar vernacular name. The present study was conducted with an aim of developing standard marker for proper identification of raw materials. In this investigation, the parameters adopted for identification of seeds of *A. graveolens* include physico-chemical values, fluorescence analysis and powder microscopy to develop their chemical parameters and diagnostic microscopic characters.

MATERIALS AND METHODS

Plant materials: Seeds of *A. graveolens* were collected from Baidyanath Pharmaceuticals, Patna.

Powder microscopy: Seeds were shade dried and powdered separately. A few grams of powder drug was taken and mixed with the clearing agent chloral hydrate, kept for 1~3 hours, heated and washed thoroughly with distilled water. Microscopic slides were prepared separately by taking one drop of the solution with the help of a capillary and mounted with 80% glycerin to observe different types of cells. Clear drawings were outlined with their magnification using Camera Lucida.

Physico-chemical parameters: Physico-chemical values such as the percentage of total ash, acid insoluble ash, water-soluble ash, water soluble extractives and alcohol soluble

extractives and moisture content were calculated as per The Ayurvedic Pharmacopoeia of India.[3]

Fluorescence analysis: Fluorescence analysis is a useful method for identification of authentic samples.[16] For fluorescence analysis, the seeds species were dried at room temperature and were coarsely powdered. The powders were studied initially under daylight and under ultraviolet radiation. Later, about 1gm of the seed powder was soaked with 10ml of various solvents like water, hexane, chloroform, acetone and methanol; alkaline solutions like aqueous and alcoholic 1N sodium hydroxide; acids like 1N hydrochloric acid and 50% sulphuric acid and was left overnight. The next day the residue was removed and the filtered solution was examined initially under daylight and ultraviolet radiations in a dark room for their characteristic fluorescent properties. The data pertaining to colour was recorded.[17] The ultraviolet lamp with transmitting radiation at wavelength of 254 nm and 366 nm units was used in this study. Standard texts,[18] were referred and the methodology of Kokoski et al,[19] was followed.

RESULTS

Powder microscopy: Powder microscopy of fruits of *A. graveolens* (Fig.1) showed fragments of indistinct, colorless cells of epicarp which bear small protuberances in side view and occasional presence of stomata, circular in outline; cells with numerous dark brown fragments of vittae composed of fairly large cells, polygonal in surface view, the cells mainly thin walled but slight thickening at the corners; innermost layer of mesocarp composed of fairly large, yellowish brown regularly arranged elongated cells with their long axes more or less parallel to one another in surface view with moderately thickened walls and usually found associated with the fragments of vittae; groups of sclereids of the mesocarp varying in shape from ovoid to slightly elongated cells with thick walls, marked with pits; numerous oil globules, group of parenchymatous cells containing numerous oil globules; prisms of calcium oxalate crystals; small thin walled fibers and vessels; groups of thick walled endosperm cells containing many oil globules, small aleurone grains and micro rosette crystals of calcium oxalate.

Physico chemical parameters: Physico-chemical values such as the percentage of total ash, acid-insoluble ash, water and alcohol soluble extractives and moisture content were calculated and are depicted in Table 1.

Table 1: Per cent age of ash and extractives of seeds of *Apium graveolens* Linn.

Parameters	*Graveolens* (%)
Total ash	9.487
Acid-insoluble ash	0.969
Water-soluble extract	89.576
Alcohol- soluble extract	92.696
Moisture content	9.612

From the above data it was observed that percent age of alcohol and water soluble extractives are 92.69 and 89.57% respectively. Total ash and acid-insoluble ash content is found to be 9.48 and 0.96 respectively.

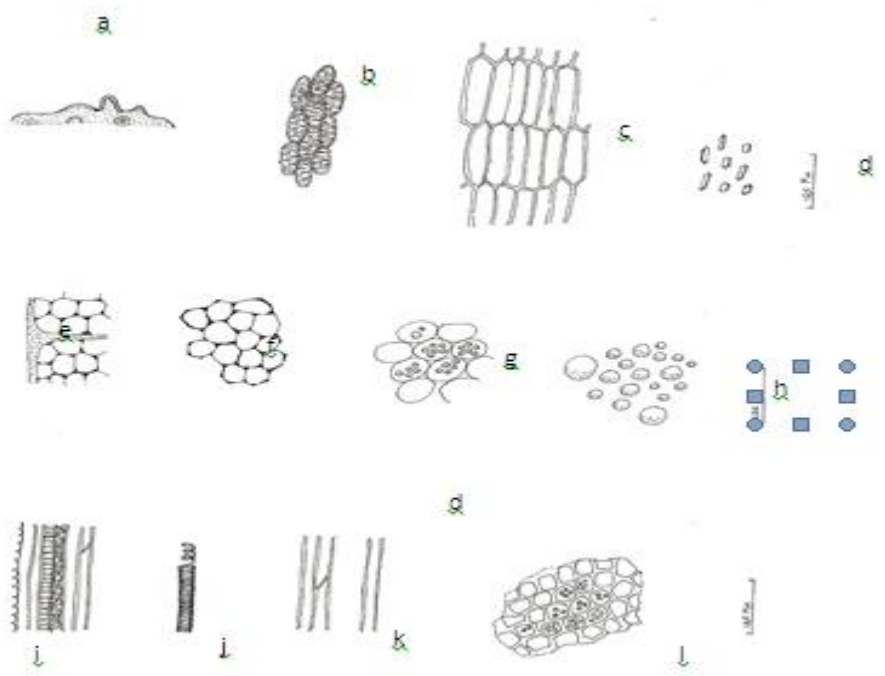

Fig. 1: *Apium graveolens* (40X).

a) Part of epicarp in side view with protuberances, b) Group of sclereids of the mesocarp, c) Part of the innermost layer of mesocarp, d) Crystals of calcium oxalate, e) Part of vitta showing transverse septa, f) Fragment of a vitta, g) Parenchyma cells containing fixed oil globules, h) Fixed oil globules, i) Fibro vascular tissue, j) Spiral vessel, k) Fibres, l) Endosperm.

Fluorescence Properties: The characteristic fluorescence properties or colours recorded through this study are depicted in Table 2.

Table 2: Fluorescence properties of seed powder of *A. graveolens* under daylight and under ultraviolet radiation.

Sl. No.	Tests	Colours observed under day light	Colours observed under ultraviolet radiation (366 nm)
1.	Seed powder	Light brown	Light brown
2.	Seed powder treated with n-hexane	Pale yellow (deep)	Peach
3.	Seed powder treated with chloroform	Greenish yellow (light)	Brownish pink
4.	Seed powder treated with methanol	Greenish yellow (light)	Light surf
5.	Seed powder treated with acetone	Greenish yellow	Tea (brown)
6.	Seed powder treated with 1 N NaOH	Yellowish orange (deep)	Milky blue
7.	Seed powder treated with 1 N HCl	Light yellow (straw yellow)	Yellowish brown
8.	Seed powder treated with H_2SO_4 (50%)	Black tea	Deep blue

DISCUSSION

Application of powder microscopy and fluorescent properties or colours recorded in this study could be used as a valuable aid in the identification of seeds in their crude forms for proper utilization. The results obtained from the microscopic study showed that the presence of numerous oil globules. Physico-chemical analysis showed total ash of 9.48% and acid insoluble ash of 0.96%, suggesting its greater medicinal effects without side effects. Similarly, some colour under day light and UV radiations were observed in fluorescence analysis which are helpful to identify the species.

REFERENCES

1. Subramoniam A., Ajikumaran Nair S. Application of apoptosis in the search for plant-derived chemopreventive/therapeutic agents. Amala Research Bulletin, 2004: 24: 1-11.
2. Shaik Imam, Hussain SJ. Some important herbs used in the treatment of cancer: part-1. In: Role of biotechnology in medicinal and aromatic plants. I A Khan & A Khanum (eds) Ukaaz Publications, Hyderabad, 2004: 11: 1-50.
3. Anonymous, Pharmacopoeia of India (The Indian Pharmacopoeia), 2nd Edn. Manager of Publications, New Delhi, 1966.
4. Anonymous, The Wealth of India, Raw Materials, CSIR, New Delhi, 1985.
5. CDRI, Lucknow and Publications and Information Directorate, New Delhi, 613.

6. Rastogi, Ram, Mehrotra, B. N., Compendium of Indian Medicinal Plants, Vol. III, CDRI, Lucknow and Publications and Information Directorate, New Delhi, 1980-1984; 575.
7. Rastogi, Ram., Mehrota, B. N., Compendium of Indian Medicinal plants. Vol. V, CDRI Lucknow and National Institute of science and communication, 1990-1994; 757.
8. Yang, Y.; Wel, S.; Mengjie, Z. and Guixing, R. Phenolic composition and Antioxidant activities of celery Cultivars. J. Food. Sci., 2010; 75(1): 9-13.
9. Takashi, Y.; Naoyuki, D.; Miki, T.; Yasuhiko, K.; Hideto, S.; Hidehara, K. and Airo., T. Preilly alcohol inhibits human breast cancer cell growth in vitro and In vivo. Breast Cancer Research and Treatment, 2004; 84: 251-260.
10. Miean, K. H. Mohammed, S. Flavonoid (Myricetin, Quercetin, Kaempfrol, Luteolin and Apiganin) content of edible tropical plants. J Agric Food Chem., 2001; 49: 3106-3112.
11. Yin, F.; Giuliano, A.E.; Law, R.E. and Vanlterle, A. J. Apigenin inhibits growth and induced G2/M arrest by modulating cyclin –CDK regulators and ERKMAP Kinase activation in breast carcinoma cells. Anticancer. Res., 2001; 21: 413-420.
12. Hamza, A. and Amin, A. Apium graveolens modulates Sodium Valproata-Induced reproductive Toxicity in rats. Journal of experimental Zoology, 2007; 307A: 1-8.
13. Pei-Wen, Z.; Lien-Chai, C. and Chun-Ching, L. Apigenin induced apoptosis through 33-dependent pathway in human cervical carcinoma cells. Life Sci., 2005; 76: 1367-1379.
14. Keil, C.; Peterman, E. and Oei, S.L. Tannins elevate the level of poly (ADP-ribose) in Hela cell extracts. Arc. Biochem. Biophy, 2004; 425: 112-121.
15. Dallak, M. Camel's Milk Protects against cadmium chloride- induced hypo-cromic microcytic anemia and oxidative stress in Red blood cells of white albino rats. Americ. J. Pharma. Toxic, 2009; 4(4): 134-141.
16. Tyler, V, E., Brady, L .R.ss, Robbers, J. E. Pharmacognosy. Lea & Febiger, Philadelphia, 1976.
17. Selvam, A.B.D. Fluorescence analysis on the powdered aerial parts of *Alternanthera philoxeroides* (Mart.) Griseb under ultraviolet radiation. J. Econ. Taxon. Bot., 2005; 29(1): 109-112.
18. Denston, T.C. A textbook of Pharmacognosy. Sir Issac Pitman & Sons, Ltd, London, 1946.
19. Kokoski, C.J., Kokoski, R.J., Slama, F.J. Fluorescence of Powder Vegetable Drugs under Ultraviolet Radiation. J. Amer. Pharma. Asso. XL, 1958; VII(10): 715-717.