International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Anatomical and Pharmacognostic Features of *Mangifera indica* Young Fruits (Avaakai) Seed Kernel

A. Bharathi[1], S. Rajan[2]

PG and Research Department of Microbiology, M. R. Government Arts College, Mannargudi, Tamilnadu, India

Abstract: ***Mangifera indica tender fruits seed kernel (MITSK) showed good therapeutic value. Pharmacognostical and anatomical studies of these plant materials showed the quality of raw material used for the medicinal purpose. Anatomy of this study material showed soft, thin papery testa and flat, planoconvex cotyledons. These are small, less prominent vascular bundles are seen in the out sarcotesta regions. Parenchyma cells with dense accumulation of starch grains and secretary cavity seen in the powder. Isolated starch grains are either circular ovoid or elliptical in shape. Other unique shape of starch grains are also seen in the powder. Good powder of MITSK was greyish yellow in colour, bitter taste and aromatic odour. It also showed effective physiochemical properties. Effective photochemical like flavonoids, tannins, phenolic compound are found in the extracts of seed kernel. This study provides scientific data to determine standards for the plant materials.***

Keywords: *Mangifera indica*, tender fruit, seed kernel, MITSK, Pharmacognosy, Phytochemistry

1. Introduction

Indian System of Medicine (ISM) utilizes varieties of herbs as a remedy for the treatment of disease. Traditional healers of indian village utilizes medicinal plants like *Mangifera indica* tender seed kernel (MITSK). Tender fruit of *Mangifera indica* along with immature fruit (Aavakai) is used to prepare pickle in India. Masuad Parvez [1] and Rajan et al.,[2] indicated the uses of MITSK in diarrhoea, dysentery, haemorrhages, haemorrhoids, diabetes, heat burn etc.,. *Mangifera indica* seed kernel (MISK) is one of the most important ingredients in siddha and ayurvedha preparations. Safety in Indian system of medicine needs to be established to make sure the safe use of medicines. Hence this study was undertaken to screen microscopical and pharmacognostical standards of MITSK. The present study will help in identifying the correct raw material used for preparing medicines.

2. Materials and Methods

Collection and Authentication of Plant Material

The MITSK were collected from the Perabalur district, Tamilnadu, India. Only Nelum variety MITSK was collected during the month of March. The plant materials were authenticated by Professor Dr. John Britto, Taxonomist, Department of Botany, St. Joseph's College, Thiruchirapalli, India. The Seed kernel was collected by cutting immature fruit of *Mangifera indica* using sterile knife, shade dried and then milled into coarse powder by a mechanical grinder.

Processing of Specimen for microscopy

MITSK was cut and removed from the plant and fixed in FAA (Farmalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure[3]. The sections were stained with Toluidine blue as per the method published by O'Brien et al.,[4]. Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections as well as clearing of seed kernel with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid[5] were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaoH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [6].

Licensed Under Creative Commons Attribution CC BY

Organoleptic Evaluation
Organoleptic evaluation refers to evaluation of the formulation by color, odor, taste, texture, etc. The organoleptic characters of the samples were evaluated based on the textual methods [7].

Physicochemical Parameters:
The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, foreign matter were analyzed by the methods given in the ayurvedic Pharmacopoeia of India[8, 9].

Fluorescence Analysis:
Fruit pulp powder were subjected to analyze fluorescence features under ultra violet light and day light after giving treatment for 48 hours with various chemical and organic solvents like ethanol, 50% sulphuric acid, 10% sodium hydroxide and dilute hydrochloric acid[10,11].

Qualitative Phytochemical Screening:
Freshly prepared *MITSK* extracts were tested for the presence of phytochemical constituents using standard textual methods [12, 13].

Microbial Limit Assay:
Dissolved 1gm of powdered plant material in 10mL of distilled water. It was serially diluted using phosphate buffer as diluent. The sample was inoculated in Nutrient agar by pour plate, Rose Bengal agar and SS agar by spread plate techniques for Bacteria, Fungi and *Salmonella* respectively. For bacteria, the plates were incubated at 37°C for 48 hrs and for fungi; the plates were incubated 25°C for 96 hrs[13].

3. Result and Discussion

Traditional heritage is one of the most important factors among countrymen. Use of medicinal plants as a healer of any cause, which is one of the rich heritages among countrymen. It is followed as grandmas remedy among the peoples of India especially Tamil nadu. Tender mango or avakkai is used for the preparation of pickles along with seed kernel (Whole tender fruit). It improves digestability and act as a good antimicrobial agent. It also enhances immunity of an individual. Pharmacognostical study will contribute in determining characteristic features of standard and raw materials. Standardization of herbal drug is a topic of great concern recently. They are subjected to variability due to its origin from heterogeneous sources.

Microscopy
Seed: The Seeds are large, ovoid and oblong in shape, compressed. Testa is Thin and papery and exabuminous. The two cotyledens are planoconvex, often unequal in size the radicle is slightly curved upward (Fig 1).

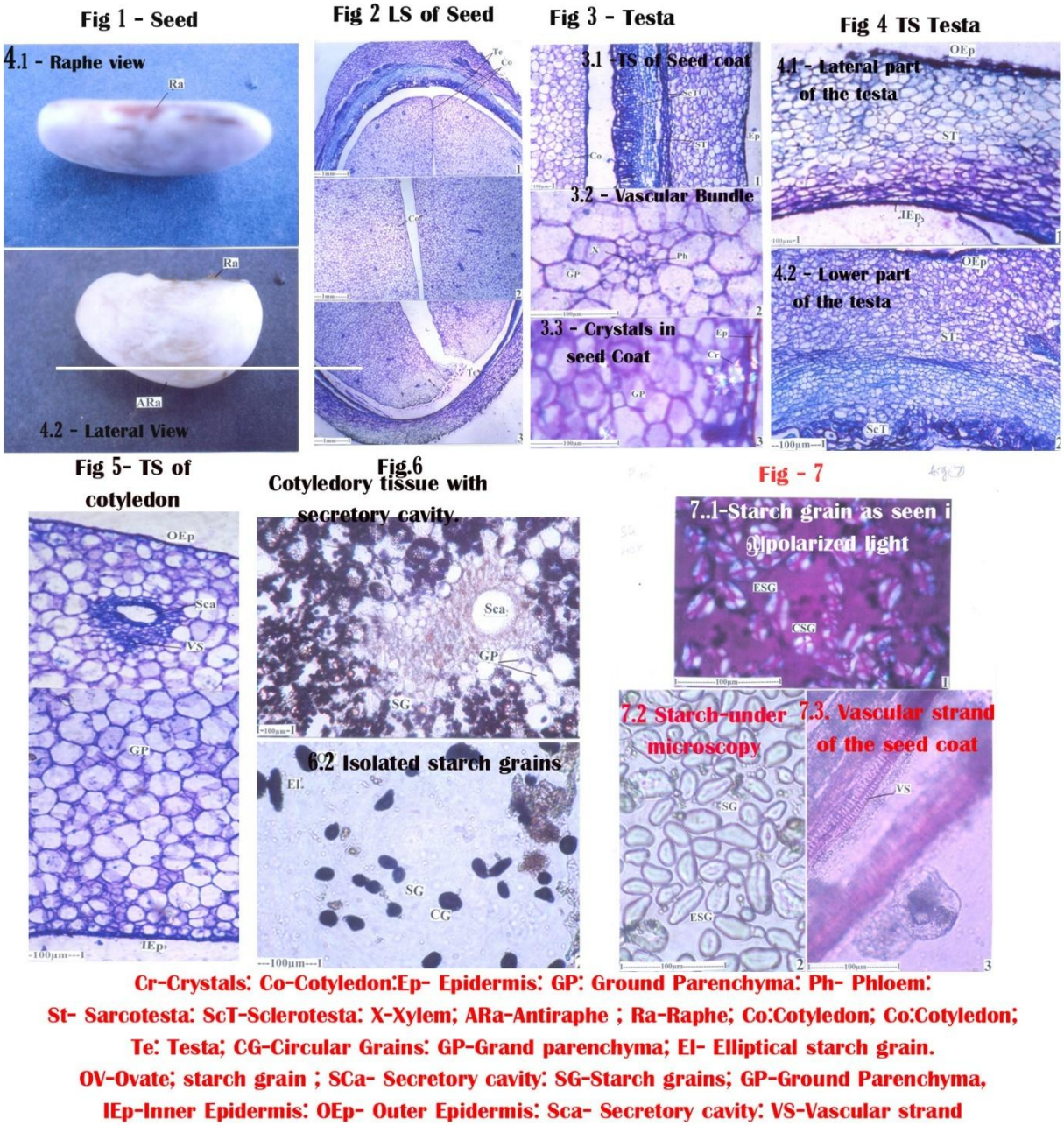
Anatamy of the Seed
Longitudinal sections, the seed shows soft, thin papery testa and flat, planoconvex cotyledons which are just opposed with their inner flat surface. The testa is thicker at one end thin at the opposite end. The testa is hetero cellular with different tissue layers. The cotyledons are homocellular with uniform cell type (Fig. 2).

Testa
The testa or the seed coat encloses the cotyledons all along circumference. The thickness and structure of the testa differ along different regions. The testa at the chalazal (upper) end is 600µm. thick. The testa is thin region consists of epidermal layer, two zones of sarcotesta. One zone being outer and another zone is middle (Fig 3.1). The sarcotesta is parenchymatous and the cells are elliptical, thin walled and compact. The sclerotesta is the inner part of the testa and in between the two zones of sacrotesta. The cells of sclerotesta are sclerenchymatous and thick walled (Fig-3.1) These are small, less prominent vascular bundles are seen in the out sarcotesta regions (Fig 3.2) The xylem elements are only two or three in the vascular bundle and phloem elements are also less in number. Calcium oxalate crystals are sparsely distributed in the sacrotesta , especially in the epidermal and subepidermal cells (Fig-3.3). The crystals are druses. Lateral part of the testa is 500µm thick. The structure is simple. It consists of outer and inner epidermal layers, more and less with similar cells. The ground tissue is parenchymatous. The cells become gradually smaller and thick walled towards the inner epidermis (Fig 4.1). The testa of the lower part is thicker than the lateral part. The lower part of the testa consists of a thin single layer of darkly stained cells. The sarcotesta is wide and parenchymatous. There is a thin layer of small slightly thick walled cells in the medium part of the teste. The inner part of the testa includes four or five layers of thickwalled and lignified sclerotesta (Fig 4.2)

Cotyledon (Fig 5)
The cotyledon has thin outer epidermis and slightly thicker inner epidermis. The ground tissue includes wide circular or polygonal compact thin walled parenchyma cells. Wide circular secretary cavities are frequently seen in ground parenchyma. The cavity is surrounded by thick walled vascular tissue or thin walled parenchyma cells.

Powder Microscopy
The powder preparation of the seed kernel contains Parenchyma cells with dense accumulation of starch grains and secretary cavity seen in the powder (Fig 6.1). The secretary is circular and 130µm wide. It is surrounded by small parenchyma cells. The ground cells around the cavity have dense deposition of large starch grains. The starch grains appear dark when stained with IKI (Fig-6.2) isolated starch grains also seen in the powder. Isolated starch grains are either circular ovoid or elliptical in shape. Other unique shape of starch grains are also seen

Licensed Under Creative Commons Attribution CC BY



Cr-Crystals: Co-Cotyledon:Ep- Epidermis: GP: Ground Parenchyma: Ph- Phloem:
St- Sarcotesta: ScT-Sclerotesta: X-Xylem; ARa-Antiraphe ; Ra-Raphe; Co:Cotyledon; Co:Cotyledon;
Te: Testa; CG-Circular Grains: GP-Grand parenchyma; EI- Elliptical starch grain.
OV-Ovate; starch grain ; SCa- Secretory cavity: SG-Starch grains; GP-Ground Parenchyma,
IEp-Inner Epidermis: OEp- Outer Epidermis: Sca- Secretory cavity: VS-Vascular strand

in the powder (Fig-6.2). Under polarized light microscope the starch grains appear as bright white bodies under dark background. They exhibit the polarimarks very clearly. In the circular type of starch grains, the white polary mark is + shape. In the elliptical starch grains the polary mark is Y shaped. In the former case the hilum is in the centre: the elliptical grain the hilum is excentric (Fig-7.1). Pollen grains as seen bright field (normal) light they appear colourless, bent their shape and size are clearly visible (Fig 7.2). They are circular, elliptical, ovate or slightly bent in shape.

Vascular strand (Fig 7.3)

Small pieces of the cotyledons are seen as a fragment in the powder. The fragment contains xylem elements which is longitudinal view. The xylum is protoxylem and the elements have annular and spiral thickenings.

Organoleptic characters

Good powder of *MITSK* was greyish yellow in colour, bitter taste and aromatic odour and smooth texture (**Table 1**).

Table 1: Organoleptic Characters of *MITSK* Powder

S. No	Test	Result
1	Colour	Greyish Yellow
2	Odour	aromatic odour
3	Taste	Bitter taste
4	Size	Length-3.6 cm; Width- 1.6 cm
5	Texture	Smooth

Physicochemical characters

Physiochemical characters of the sample were recorded in table 2. It was observed that total ash value of MItSK was 1.85%. Higher extractive matters were collected using ethanol (12.6%) followed by ethanol (10%).

Licensed Under Creative Commons Attribution CC BY

Table 2: Physicochemical Parameters of *MITSK* Powder Parameters

S. No	Test	Result
1	Foreign matter	<0.1%
2	Dry Powder Particle size	0.24 µm
3	Wet powder Particle size	0.28 µm
4	Foaming index	432.1U
5	Swelling index	2.6 %
6	Acid insoluble ash value	1.95%
7	Water soluble ash value	1.5 %
8	Total ash	1.85%
9	Water extractive	10%
10	Alcoholic extractive	12.6%

Phytochemical analysis

Powder and extract was subjected to qualitative chemical tests and the results were presented in Table 3. The results revealed the presence of flavanoids, tannins, lignins and phenolic compounds in both extracts. Terpenoids are absent in aqueous extract but indicated in ethanolic extract. Terpenoids showed antiseptic and antihelmentic[14]. Phenolic compounds are used in the treatment of burns as they precipitate the proteins of exposed tissue to form a protective covering [15]. They are also used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote [16].

Table 3: Qualitative Phytochemical Analysis *MITSK* Extracts

S. No	Test	Aqueous extract	Alcoholic extract
1	Alkaloids	Negative	Negative
2	Steroids	Negative	Negative
3	Terpenoids	Negative	Positive
4	Flavonoids	Positive	Positive
5	Saponins	Negative	Negative
6	Phenolic compounds	Positive	Positive
7	Tannins	Positive	Positive
8	Lignin	Positive	Positive
9	Phlabo tannins	Negative	Negative
10	Fat and Oil	Positive	Positive
11	Inulin	Negative	Negative
12	Cardiac glycosides	Positive	Negative
13	Proteins	Negative	Positive
14	Carbohydrates	Positive	Positive

Fluorescence analysis

Diferent colours of chromophores were produced when the drug powder is treated with different chemicals. Black coloration was noted after 48 hrs of H_2SO_4 treatment under visible light. These chromatophoric colors under UV and visible light illustrated the nature of raw materials (**Table 4**). Fruit pulp exhibited characteristic colors when treated with different chemicals that may be due to chromatophores present in the powder. This may help to assess the purity of the drug [17].

Table 4: Fluorescence Analysis of *MITSK* at 24 hours

S. No	Test Plant Powder +	Day Light	UV Light
1	Chloroform	Orange	Greenish yellow
2	Hexane	Light Brown	Greenish yellow
3	Benzene	Red	greenish yellow
4	Aqueous NaOH	Dark red	Dark Brown
5	Alcoholic NaOH	Dark red	Greenish yellow
6	1NHcl	Red	Yellowish red
7	Ethanol	Sandal	greenish yellow
8	Ethyl acetate	Pale yellow	Greenish yellow
9	Acetone	Dark brown	greenish yellow
10	50% H_2SO_4	Magenta	Dark pink

Microbial Limit assay: Total bacterial load available in the MITSK powder was within the limits of ayurvedic pharmacopeia of India. Only $3X10^2$ CFU of bacteria and No funguses were isolated per gram of plant powder. Plant powder was free from enteric bacteria like *Escherichia coli*, *Salmonella sp.*, and *Shigella sp*.

MITSK is used in the ISM for the treatment of gastrointestinal infections [18,19,20,21,22]. The present study provides good information to check the identity of the MITSK. This is a first of its kind of work on record about the MITSK. Standards determined in the present work would be useful to detect the genuinity of this medicinally useful part. Low ash value (1.85%) indicates the purity of the seed kernel. Ash values were used to determine the quality and purity of crude drug. Inorganic elements present in the raw drug indirectly indicate the water soluble ash [23]. The extractive values were useful to evaluate the chemical constituents present in the crude drug[23]. Ethanol-soluble extractive value was higher, indicting the presence of polar chemicals such as flavanoids. Ethanol extractive value showed the presence of phenolic compounds. Presence of phenolic compounds and tannins in this plant part supports chemically the antidiarrheal activity and anti microbial activity, which was in agreement with various earlier reports [18,19,20,24,25]. Behaviour of drug materials under UV radiation and visible light exhibited different colour depending up on the various chromophores present in the material. The same extract may appear different at different wavelength of light [23,24]. Seed kernel powder exhibited black coloration when treated with sulphuric acid. The results revealed the presence of alkaloids, terpenoids, flavanoids, and tannins in aqueous extract. Preliminary phytochemical tests were helpful in predicting the nature of drug and also useful for the detection of different constituents present.

4. Conclusion

Shape of the selected seed kernel was like developing chick embryo. Cotyledons of the MITSK found to be outer white and inner surface was pale pink. The transverse section showed upper and lower epidermis, many cortical cells, which are compactly, arranged parenchyma cells and fibres were also noticed. Colour of the MITSK was found to be brownish yellow with mild aromatic odour and Bitter taste. Physiochemical parameters are within the standards of ayurvedic pharmacopeia. Forein matter also is <0.1%. Microbial load also with in pharmacopeal limits.

References

[1] Masuad Parvez, G.M. Pharmacological activities of Mango (*Mangifera indica*): A Review. Journal of Pharmacognosy and Phytochemistry. 2016. 5(3): p1-7.
[2] Rajan, S, Surya, D, Vijaya, P, Prabhu, K, and Thirunalasundari, T. Pharmacognostical and phytochemical studies of *Mangifera indica* seed kernel.

Licensed Under Creative Commons Attribution CC BY

Journal of Pharmacy Research . 2011. 4(11): p4272-4275

[3] Johansen, DA. 1940. Plant Microtechnique. Mc Graw Hill Book Co; New York. Pp.523.

[4] O'Brien, TP, Feder, N and Mc Cull, ME. Polychromatic Staining of Plant Cell walls by toluidine blue-O. Protoplasma. 1964. 59: p364-373.

[5] Sass, J.E. Elements of Botanical Microtechnique. McGraw Hill Book Co; New York. 1940. pp.222.

[6] Easu, K. Plant Anatomy John Wiley and sons. New York. 1964. pp.767.

[7] Siddiqui A and Hakim MA. Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, (appendix), 24-25 January. New Delhi: Central Council for Research in Unani Medicine (CCRUM); 1995.

[8] Wallis, TE. Textbook of Pharmacognosy, 5th ed, CBS Publishers and Distributors, New Delhi, India; 2005.

[9] Anonymous. Quality Control of Medicinal Plant Materials. (An authorized publication of World Health Organization, Geneva) New Delhi: A.I.T.B.S. Publishers & Distributors India, 2002.

[10] Kokate, CK. Practical Pharmacognosy, 1st ed, Vallabh Prakashan, New Delhi 1986.

[11] Chase, C.R, and Pratt, R.S. Fluorescence of Powdered Vegetable drugs with particular reference to Development of a System of Identification. Journal of American. Pharmacology Association. 1949. 38: p32.

[12] Harborne, JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Champman and Hall, 3rd ed. New York, USA: 1998.

[13] Anonymous: Indian Pharmacopoeia, Vol. I & II. Government of India, Ministry of Health and Family Welfare, the Controller of Publications, Civil Lines, Delhi– 110054, 1996.

[14] Riyanto, S.S, and Mustafa, A.L. Isolation of some chemical compounds from *Aegle marmelos*. Majalah Farmasi Indonesia 2000. 11(1): p17-32.

[15] Handa, S.S., Sharma, A, and Chakraborti, K.K. Natural products and plants as liver protecting drugs. Fitoterapia. 1986. 57: p307-45.

[16] Gopiesh, K.V., and Kannabiran, K. Larvicidal effect of *Aegle marmelos*, *Gymnema sylvestrre* and *Eclipta prostrate* against *Culex qinquifaciatus* mosquito larvae. African Journal of Biotechnology. 2007. 6(3): p307-311.

[17] Kokate, C.K. Practical Pharmacognosy, Vallabh Prakashan, New Delhi, India. 1994.

[18] Sairam, K, Hemalatha, S, Ashok Kumar, T, Srinivasan T, Jai Ganesh, M, Shankar, M, and Venkataraman, S. Evaluation of anti-diarrheal activity in seed extracts of *Mangifera indica*. Journal of Ethnopharmacology. 2003. 84(1): p11-15.

[19] Rukmini, C, and Vijayaraghavan, M. Nutritional and toxicological evaluation of mango kernel oil. Journal of American oil chemical society. 1984. 61: p780-792.

[20] Ramteke, R.S, Vijayalakshmi, M.R, and Eipeson, W.E. Processing and value addition of mangoes, Indian food industry. 1999.18: p155-163.

[21] Keher, and Chanda. Herbal Medicine, Current science. 1946. 15: 48.

[22] Kirtikar, K.R, and Basu, B.D. Indian Medicinal Plants, Lalit Mohan Basu, Allahabad, India, 1956

[23] Kokate, CK. Practical Pharmacognosy, 1, 1, Vallabh Prakashan, New Delhi, 1986, pp15-30.

[24] McDonald, S, Prenzler, P.D, Autolovich, M, and Robards, K. Phenolic content and antioxidant activity of olive extracts, Food chemistry. 2001. 73: p73-84.

[25] Toshihide, K, Hadjime, N, Megumi, A, Shigeko, U, Yoshiharu, K, and Shun'ichi, D, Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L.) kernel seeds. Food chemistry. 2000. 71(1): p61-66.

[26] Chase, C.R, and Pratt, R.S. Fluorescence of Powdered Vegetable drugs with particular reference to Development of a System of Identification, Journal of American Pharmacology Association. 1949. 38: 32.

Licensed Under Creative Commons Attribution CC BY