



IJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 Issue: VII Month of publication: July 2017

DOI:

www.ijraset.com

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Effect of Extraction Variables on the Extraction of Colourant from Leaves of Aegle Marmelos

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Abstract: Human beings have been using herbs and plants for curing various ailments and diseases since ancient times due to their medicinal values and easy availability in nature. Later with advancements in technology these plants and herbs got major importance in pharmaceutical industry due to their suitable chemical composition, abundant availability and easy access. Aegle Marmelos (Bael) is such a type of plant which is found throughout in the forests of India. The leaves and fruits of this plant are used in worshipping the lord Shiva. But no or very less literature was found on the colouring behaviour of the leaves of this plant. Present work focuses on the effect of extraction parameters on the extraction of colourant from leaves of aegle marmelos. MLR (Material to Liquor Ratio), temperature, time and pH were varied to check the effect on colour strength. Colour strength of the liquid was checked on computer colour matching software (CCMS). In the study it was observed that these parameters have significant effect on the extraction efficiency. The optimised extraction conditions were MLR 1:30, temperature 90 °C, time 90 minutes and pH 8. Natural dyes are renewable, biodegradable and eco-friendly in nature. Thus, the extract obtained has potential to be used as sustainable natural dye for colouration of textiles.

Keywords: Aegles Marmelos, Leaves, Extraction, Temperature and Time

I. INTRODUCTION

Textile and apparel sector plays pivotal role in global economy but it causes serious environmental hazards. There is dire need to replace toxic synthetic chemicals used in this sector with eco-friendly alternatives. In case of textile wet processing certain plant extracts [1] can be used as natural dyes. Natural dyes have poor affinity to textiles so biopolymers can also be used as mordants and finishing agents simultaneously [2, 3]. Studies carried out by different researchers show that colouring agent can be successfully extracted by varying the extraction conditions [4] and the extract can be used as natural dyes for dyeing of textile with moderate to good washing fastness [5].

Aegle marmelos belonging to family Rutaceae, is commonly known as Bael in indigenous systems of medicine and has been regarded to possess various medicinal properties. The bael is one of the sacred trees of the Hindus. Leaves are offered in prayers to Lord Shiva and Goddess Parvathi since ancient times [6]. Bael is a deciduous sacred tree associated with Gods having useful medicinal properties, especially as a cooling agent. This tree is popular in Shiva and Vishnu temples and it can be grown on every house. Its leaves are trifoliolate symbolising the thrimurthies-Brahma, Vishnu, Shiva, with spear shaped leaflets resembling thrisoolam the weapon of Lord Shiva. Many legends, stories and myths are associated with this tree. In India flowering occurs in April and May soon after the new leaves appear and the fruits ripens in 10-11 months from bloom March to June of the following year [7, 8]. Various parts of the plant consists different chemical constituents which make it wide applicable in ayurveda and pharmaceutical industry. The medicinal properties present in Aegles Marmelos may be used in sustainable dyeing and finishing of textiles. Therefore in present investigation, leaves of Aegles Marmelos were extracted and extraction conditions were optimised for maximum colour extraction in terms of obtained absorbance value. The foregoing section discusses plant profile, botanical description, chemical constituents, traditional uses and optimisation procedure of Aegles Marmelos

A. Plant Profile

1) Scientific Classification [9] :

- a) Kingdom: Plantae
- b) Order: Sapindales
- c) Family: Rutaceae
- d) Subfamily: Aurantioideae
- e) Genus: Aegle
- f) Species: Aegle Mannelos
- g) Botanical Name: Aegle marmelos

2) *Vernacular Names [10]:*

- a) *English:* Bengal quince, Beal fruit, Golden apple, Indian quince, Stone apple.
- b) *Tamil:* Ahnigam, Iyalbudi, Kuvilam, Matilangai, Vilwam, Villuvam.
- c) *Telugu:* Bilvamu, Maluramu. Maredu, Sriphalamu
- d) *Hindi:* Bel, Bill, Simhal, and Bela,
- e) *Sanskrit:* Adhararutha, Asholam, Atimangaliya, Bilva.
- f) *Bengal:* Bael, Bel,
- g) *Gujarat:* Billi,
- h) *Malayalam:* Koovalam, Vilwam.

3) *Common Names:*

- a) Bael, Bengal quince, golden apple, Japanese bitter orange, wood apple, etc.

4) *Botanical Description:*

- a) *Plant Type:* Aegle marmelos is a slow-growing, medium sized tree, up to 12 to 15 feet in tall with short trunk, thick, soft, flaking bark and spreading, sometimes spiny branches (Fig 1). Fruit, Leaf, Bark and Decoction of the bark have been used in traditional medicinal system for the treatment of various diseases. [11]



Figure 1 Aegle Marmelos plant

- b) *Soil Type:* Bael is said to do best on rich, well-drained soil, but it has grown well and fruited on the oolitic limestone. It also grows well in drastic swampy, alkaline or stony soils having varied pH range from 5 to 8. In India it has the reputation of thriving where other fruit trees cannot survive [12].
- c) *Tree Management:* The Aegle marmelos tree requires minimum amount of fertilizer and water for irrigation. The spacing in orchards is 6 to 9 m between trees. Seedlings begin to bear in 6 to 7 years, vegetatively propagated trees in 5 years. Full production is reached in approximately 15 years. Normally, the fruit is harvested when yellowish-green in colour and kept for 8 days while it loses its green tint. Then the stem readily separates from the fruit. A tree may yield as many as 800 fruits in a season [12].

5) *Chemical Constituents:*

Some of the important chemical constitute of the Aegle marmelos are as follows

- a) *Alkaloids:* The alkaloids comprise the largest single class of secondary plant substances. New alkaloids from the leaves of Aegle marmelos were reported viz., ethyl cinnamamide, O-3,3-(di methylallyl) halfordinol, N-2-methoxy-244-(3',3'-dimethylallyloxy) phenyl ethyl citunnamide, etc [14,22].
- b) *Terpenoids:* The essential oil of Aegle marmelos (L) Correa leaves were studied very much extensively in India by various workers since 1950. a-Phellandrene was found to be the common constituent of the essential oil from leaves, twigs and fruits. a-Phellandrene (56%) and p-cymene (17%) were reported from leaf oil. Later, similar report was published on leaf essential oil by many workers. P-Menth-1-en-3,5-diol was isolated and characterized from Aegle marmelos leaves. Limonene (82.4%) was reported as the main constituent from Aegle marmelos leaves and it was shown that limonene is characteristic marker for identification of Aegle marmelos oil samples [14,20].
- c) *Coumarins:* Marmelosin, marmesin, imperatorin, marmin, alloimperatorin, methyl ether, xanthotoxol, scopoletin, scoparone, umbelliferone, psoralen and marmelide has also been reported [15].

- d) *Phenylpropanoids*: These are naturally occurring phenolic compounds, which have an aromatic ring to which three-carbon side chain is attached. Among the phenylpropanoids are included hydroxycoumarins, phenylpropenes and lignans. The most widespread plant coumarin is the parent compound, coumarin itself, which occurs in over twenty-seven plant families. Marmesin was established as a new compound from leaves, which is also a constituent of heartwood and root [16].
- e) *Tannins*: The maximum tannin content in bael fruit was recorded in the month of January. There is as much as 9% tannin (4, 7, 8 - trimethoxyfuro- quinolone) in the pulp of wild fruits. [17].
- f) *Polysaccharides*: Galactose, arabinose, uronic acid and L rhamanose are obtained on hydrolysis [17,21].
- g) *Flavonoids*: Mainly includes Rutin, Flavone, flavan-3-ols, flavone glycosides [18]

The different parts of Bael tree exhibits various important pharmacological activities due to presence of aforesaid chemical constituents as shown in Table I

TABLE I
Pharmacological activities of Aegle Marmelos

Antibacterial activity[6]	Cardiotonic activity[31]
Antihistaminic activity[24]	Anxiolytic and antidepressant activity[32]
Anti-inflammatory, antipyretic and analgesic activity[25]	Myocardial infarction[30]
Hepatoprotective activity[26]	Anticonvulsant activity[34]
Insecticidal activity[27]	Wound healing activity [33]
Testicular activity[30]	Anti-stress and adaptogenic activity[35]
Hypoglycemic and antioxidant activity[28,37]	Antifertility activity[36]

6) *Traditional Uses:*

All parts of Aegle marmelos are medicinally useful like leaves, fruit pulp, flower, stem bark, root bark etc.

- a) *Leaves*: Leaves are used as mild laxative, or the inflammation of the mucous membrane having a free discharge and for asthma. The decoction of the leaves is febrifuge, or helps in eliminating fever and is an expectorant, or promotes the removal of mucous secretion from the bronchial tubes. The leaf juice is given in dropsy or the abnormal accumulation of liquid in the cellular tissue accompanied with constipation and jaundice. A hot poultice of the leaves is applied in ophthalmia or severe inflammation of conjunctiva with acute bronchitis and inflammation of the other body parts [16].
- b) *Root*: The decoction of the root and sometimes the stem bark is useful in intermittent fever, also in hypochondriasis and palpitation of the heart. The decoction of root is given with sugar and fried rice for checking diarrhoea and gastric irritability in children. Root is a one of the ingredients of Dasamoola a standard Ayurvedic remedy for loss of appetite and puerperal diseases e.g. Inflammation of uterus [13].
- c) *Flower*: Distillation of flowers yielded a drug used as tonic for stomach and intestine, anti-dysenteric, antidiabetic, diaphoretic and as local anaesthetic. It is also used in epilepsy and as expectorant [19].
- d) *Fruit*: Fruit is eaten during convalescence after diarrhea. It is valid for its mild astringency and as remedy for dysentery. The traditional healers of southern Chhattisgarh use dry powder of fruit with mustard oil for the treatment of burn cases. One part of powder and two part of mustard oil are mixed and are applied externally. Fruits are also used in gastric troubles, constipation, laxative, tonic, digestive, stomachic, brain and heart tonic, ulcer, antiviral, intestinal parasites, gonorrhoea, epilepsysion, etc [11,23]
- e) *Ripe Fruit*: The ripe fruit promotes digestion and is helpful in treating inflammation of rectum. The ripe fruit extract showed antiviral activity against ranikhet disease virus. Pulp of ripe fruit is sweet, cooling, aromatic and nutritive when taken fresh. Fruit pulp marmalade is used as prevention during cholera epidemics, also given to prevent the growth of piles, useful in patients suffering from chronic dysenteric condition characterised by alternate diarrhea and constipation relieves flatulent colic from a condition of chronic gastrointestinal problems. Fresh juice is bitter and pungent fruit extract lower the blood sugar [19,11].
- f) *Unripe Fruit*: Fine powder of unripe fruit showed significant effect on intestinal parasites and also effective against Entamoeba histolytica and Ascaris lumbricoides. Unripe fruit is used as an astringent in dysentery, stomach ache in diarrhea, tonic,

digestive, demulcent, described as cardiacal, restorative, given in piles, Decoction of unripe fruit is astringent, useful in diarrhea and chronic dysentery [13,23].

7) *Bioactive Structures and Compounds from different parts of Bael [38]:* Some of the important bioactive chemical constituents of bael is illustrated in Fig 2 where as biological activities of different parts of bael is indicated in Table II.

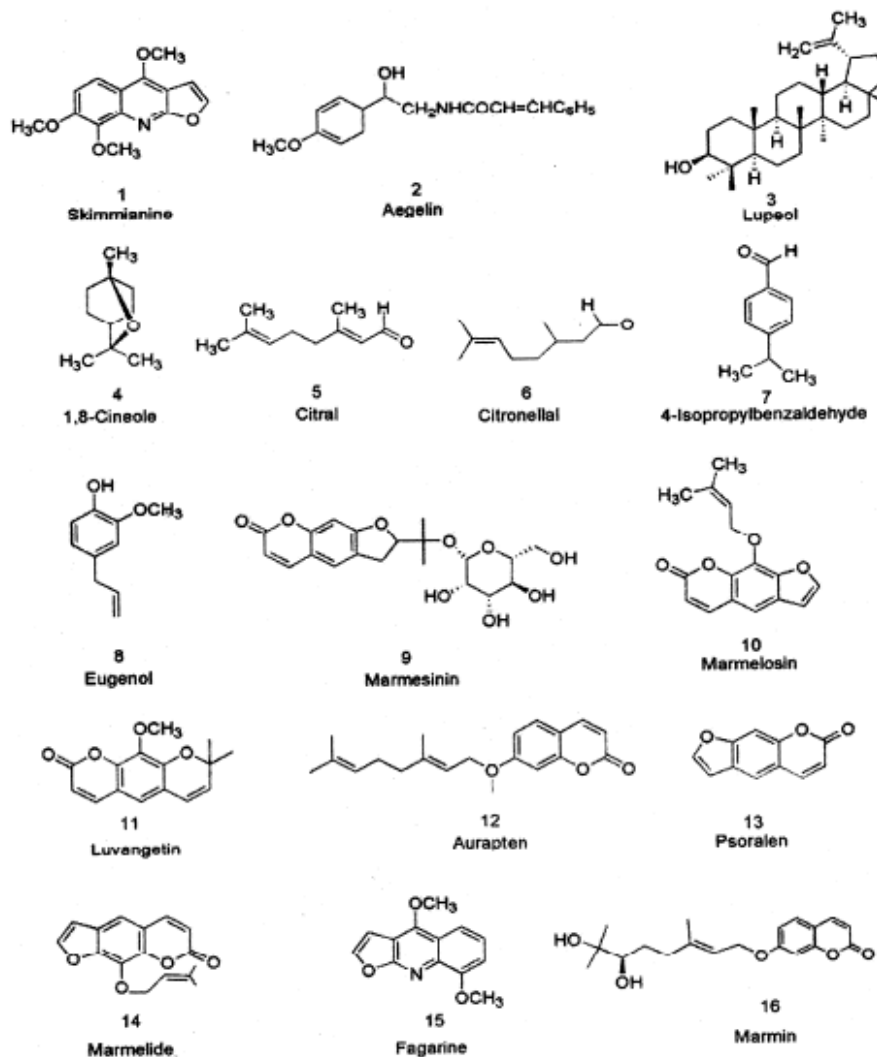


Figure 2 structures of purified bioactive compounds from bael.

TABLE II

Bioactive compounds isolated from various parts of bael [39]

Bael compound	Source	Biological activity
Skimmianine	Leaf, immature bark	Anti cancer
		Anti methamphetamine
		Sedative, hypnotic
		Analgesic
		Anticonvulsive
		Antipyretic
		Hypothermic
		Antidiuretic

		Antimalarial
Aegelin	Leaf	Cardioactive
		Antihyperglycemic
		Antidislipidemic
Lupeol	Leaf	Cardioactive
		Anti-inflammatory
Cineol	Leaf	antiulcer
Citral	Leaf	Antiallergic
		Antiseptic
Citronellal	Leaf	Antiseptic
Cuminaldehyde	Leaf	Antibacterial
Eugenol	Leaf	Antioxidant
		Antibacterial
		Hepatoprotective
		Antiulcer
Marmesinin	Leaf	Antioxidant
		Cardioprotective
Marmelosin	Fruit	Antihelminthic
		Antibacterial
Luvangetin	Fruit	Antiulcer
Aurapten	Fruit	Heart beat inhibitor
Psoralen	Fruit	Antispasmodic
		Artemicide, cytotoxic
Marmelide	Fruit	Antiviral
Fagarine	Mature bark	Abortifacient
Marmin	Immature bark	antiulcer
Tannin	Unripe fruit	Antidiarrhoea
		astringent

II MATERIAL AND METHODOLOGY

A. Material

Aegle Marmelos leaves were collected from campus of NIT, Kurukshetra, Haryana. Washing was carried to remove if any dirt present on it. Dried leaves were crushed and grounded to make fine powder. This powder was used in the study for extraction of colour. All the chemicals used in the study were of LR (Laboratory Reagent) grade.

B. Extraction of colour

Different experiments were performed to find out the optimised conditions for the extraction of colourant from dry leaves of aegle marmelos. The variables for the extraction are given in Table III. To check the effect of MLR, extraction was carried out in boiling water and MLR was optimised. The effect of temperature was checked by carrying the extraction in water by taking the optimised MLR. Further, the extraction at optimised MLR and Temperature was carried out to check the effect of pH. All the optimised variables (MLR, temperature and pH) were used to check the effect of time on extraction of colourant from the dry leaves powder of aegle marmelos.

TABLE III

Variables for the extraction of colourant from dry aegle marmelos leaves

Variables	Factors
MLR	1:20, 1:30, 1:40
pH	4, 6, 8
Time (min)	60, 90, 120
Temperature (°C)	60, 80, 100

III RESULTS AND DISCUSSION

In the present study, the extraction of natural dye from the dried leaves of aegle marmelos was done. The effect of MLR, pH, time and temperature has been studied for the extraction of dye. The following section explains the effect of each factor.

A. *Effect of MLR:* It can be observed from Fig. 3 that initially with the increase in the M:L ratio from 1:20 to 1:30 colour strength value obtained of extract increases and further with further increase in M:L ratio the colour strength decreases. When M:L ratio is less, the dye molecules are congested and mobility is less hence less colour strength. With increase in MLR mobility increases and colour strength also increases. Therefore optimum extraction is obtained at M: L ratio 1:30.

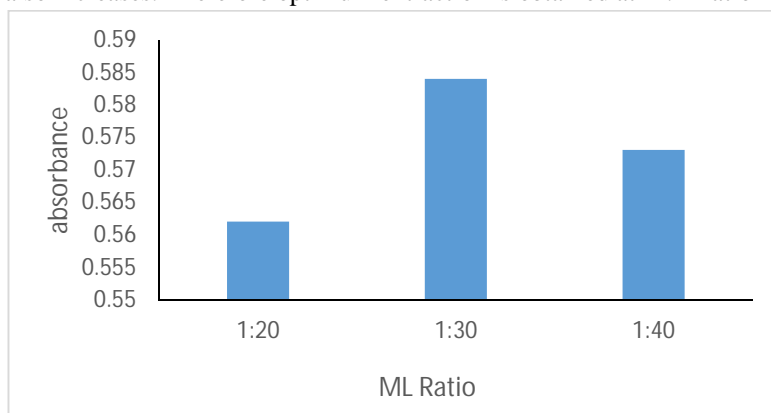


Figure 3: Effect of MLR on extraction of dye

B. *Effect of pH*

The effect of pH on colour strength of dyed wool with Aegle Marmelos extract varies with respect to control conditions. It can be depicted from the Fig 4 that maximum dye contents are extracted in alkaline medium at pH 8. Phenols are weak acids as these are having OH group in there structure. At higher pH these phenolic contents of extract transforms into their anionic forms and equilibrium yield increases with an increase in pH value.

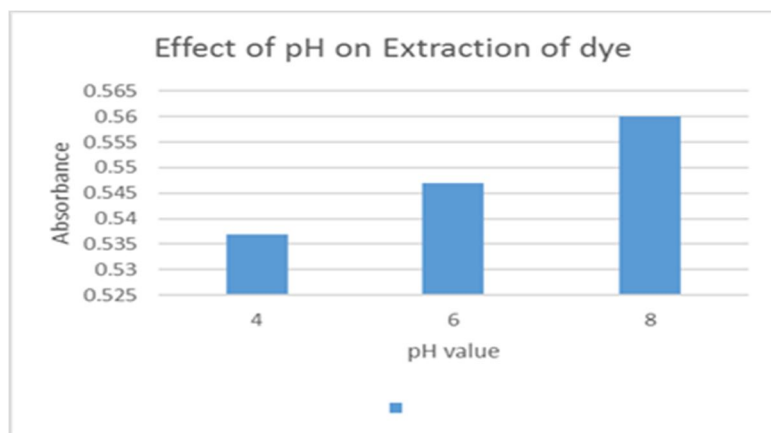


Figure 4: Effect of pH on extraction of dye

C. Effect of Temperature

The effect of temperature on natural dye extraction from aegle marmelos in terms of obtained colour strength is shown in Fig 5. It can be observed that colour strength values of extracts increases with rise in temp from 60°C to 100°C. Although at 100°C more extraction yield was obtained but the increase in extraction yield from 60°C to 100°C was very less, therefore for further extraction 60°C temperature was chosen as optimum temperature.

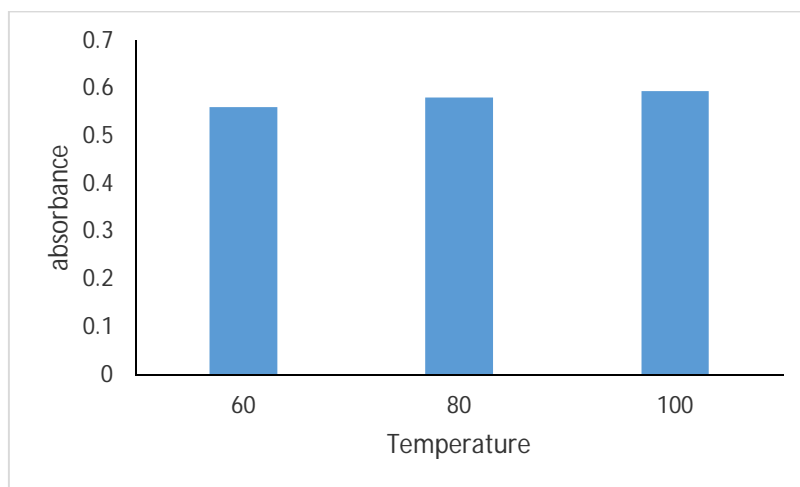


Figure 5: Effect of temp. on extraction of dye

D. Effect of Time

The effect of time on natural dye extraction from aegle marmelos in terms of obtained colour strength is shown in Fig 6. It was observed that extraction time of 90 minutes gives maximum dye extraction as there after no increase in absorbance values of dye extract.

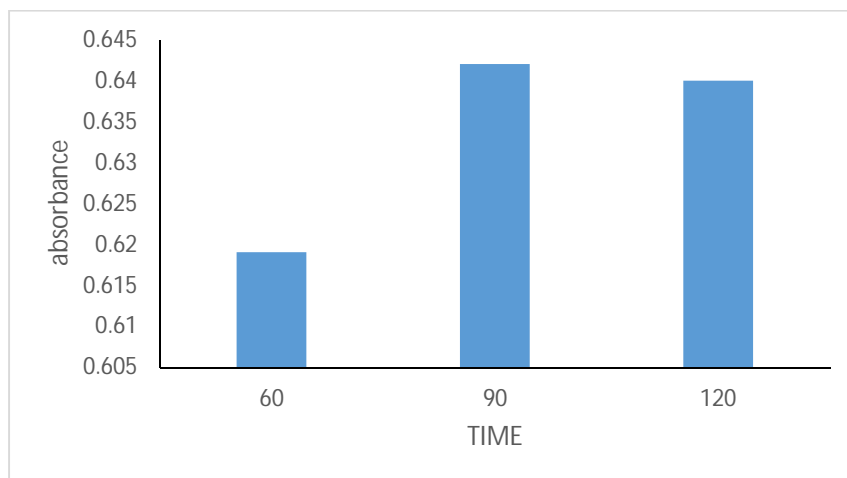


Figure 6: Effect of time on extraction of dye

IV. CONCLUSIONS

The results of the study affirm that extraction conditions play a vital role in the extraction of colour from leaves of aegle marmelos. Extraction variables have significant effect on the colour strength values of the extract so it becomes essential to optimise the extraction conditions. Optimum conditions for extraction were MLR 1:30, pH 8, temperature 60°C and time 90 minutes. It can be concluded that colour extracted from leaves of aegle marmelos have potential to act as natural dye for textiles.

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