

Behaviour Study of *Aegle Marmelos* Phytochemicals

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Abstract

The aromatic tree; *Aegle marmelos* is recognized for its spiritual and pharmacological benefits. Due to its mythological background and its biodegradable constituents, present study is focused behavioral study of *Aegle marmelos* phytochemicals in light and dark conditions and also in various solvents. Behavioral pattern quantified by TLC and UV. The change in color of reaction may augment in the chemistry of natural products.

KEYWORDS: Bilva, Flavonoids, alkaloids Ayurveda, Phytochemistry

Introduction

For health care more than 80% of world's population depends on diversified ingredients from medicinal plants that produce resistance in living beings (Ncube N.S., et.al.2008). Hence to screen natural ingredients and its activity for drugs preparations which are inexpensive, safe, biodegradable, have less side effects and have long shelf life before resistance sets. Natural products owing to their multiple medicinal properties; scientist was enlightening the attention of indigenous communities.

Aegle marmelos (Rutaceae) originated in India growing in south Himalayan range. Beside its spiritual significance, its aerial parts are used as ethno medicine against human ailments. It contains bioactive compounds such as alkaloids, coumarins, terpenoids, fatty acids and amino acids. *Aegle marmelos* exhibited Pharmacological activities (pallavi maity et. al., 2009) such as hypoglycemic activity (Sachdewa et.al., 2001; Phuwapraisirisan et. al., 2008), Antimicrobial activity (Rani et al., 2004). Anti-oxidative activity (Narayan et al., 2009). Radioprotective activity

(Mishra et.al., 2010) Anti-inflammatory activity (Arul V. et.al.,2005) Anticancer activity, (Veerappan et.al.,2007) Chemopreventive potentially (Donga et.al.,2011) Hemagglutinating activity (Jindal et. al.,2017), Aantiamoebic and antihistaminic (Baliga et al., 2010) etc.

Natural medicine is attracting more attention than allopathic system (Manish Jindal et.al., 2017) bilva, bel, sadaphal and shriphaal used for worship of Lord Shiva from historic times and *Aegle marmelos* leaves exhibited cooling effect. Hence present paper focused on the effect of sunlight, dark and oven on leaves and fruits phytochemicals.

MATERIALS AND METHODS

Collection

The *Aegle marmelos* leaves and fruits are collected from Vadagaon Budruk Pune, Maharashtra in the month of July 2016. *Aegle marmelos* leaves and fruits washed well, dried in dark, sunlight and oven dried separately. The powdered used for the detection of phytochemicals.

Preparation of Plant Extract

Dried leaves and fruits powder (50gm) from each condition were dissolved in 70% ethanol and 80% methanol (1:10)

separately. The respective extract was divided into three parts. First part of extract kept in the dark, second part of extract explored to sunlight and third part explored to daylight for 3 days at room temperature in sterilized beakers wrapped with aluminum foil to avoid evaporation. After 3 days, all extract were filtered through Whatman no.1 filter paper and kept it in incubator at 40°C till all solvents had completely evaporated from extract. The extracts were subjected to preliminary phytochemical screening. The obtained results were compared with aqua extract dried leaves and fruits powder from respective conditions and solvents.

All extracts were monitored by TLC using toluene: Acetone (8:2) solvent system. The plates were dried and visualized under normal day light, ultraviolet light (254nm & 366nm) for detection of spot.

Change in UV behavioral pattern of phytochemical from extracts were monitored on shimadzu UV-1800 spectrophotometer.

Screening of bioactive compounds from SDLP, DDLP, SDFP, DDFP, ODLP, ODFP.

Abbreviations:

SDLP- Sunlight dried leaf powder,

DDLp- Dark dried leaf powder,

SDFP- Sunlight dried fruit powder,

DDFP- Dark dried fruit powder **ODLP-**

oven dried leaf powder **ODFP-** oven dried fruit powder

Phytochemical Tests:

Reducing sugar: Deionized water extract of leaves (1gm) and fruits (1gm) was tested for reducing sugar with Fehling solution. Change in colour was noted for all extract samples.

Tannins: Tannins was tested with 5% ferric chloride by using aqueous extract

(1gm) change in colour was noted for all extract samples.

phlobatannins: Deionized water extract of **SDLP, DDLP, SDFP, DDFP, ODLP** and **ODFP** boil at 100°C and presence of phlobatannins with few drops of 1% HCl was observed.

Saponins: Deionized water extract of **SDLP, DDLP, SDFP, DDFP, ODLP** and **ODFP** was boiled for 15 min and Saponin content is determined.

Terpenoids: In deionized water extract of **SDLP, DDLP, SDFP, DDFP, ODLP** and **ODFP** chloroform (2ml) followed by addition of 3ml conc. sulfuric acid was carried out to observe the change in colour for all extract samples.

Alkaloids: In aqueous extract (100µl), 2-3 drops of Wagner's reagent added to observe orange red precipitation.

Flavonoids: presence of flavonoids was tested in deionized water extract of **SDLP, DDLP, SDFP, DDFP, ODLP** and **ODFP** by adding few drops of 1% NH₃. Change in colour was noted for all samples. In addition it was confirmed by DMSO solution. The ethanolic aqueous extract was heated followed by adding Mg and conc. HCl.

Polyphenols: Ethanolic extract and aqueous extract of **SDLP, DDLP, SDFP, DDFP, ODLP** and **ODFP** explored with folin-ciocalteu reagent and 9ml d/w. between 1-8 min. and added sodium carbonate solution (8ml) vortex. The test samples were in dark condition to avoid samples oxidation at 0°C and O.D was measured at 760nm.

Results and Discussion

Sundried, dark condition dried and oven dried powder received 30 gm, 29 gm and 27gm of from 50gm leaves while 19gm from in sundried and in dark condition and oven dried produced 16 gm from the fruits. The presence of phytochemicals in aqueous, methanol

and in ethanol solvents in SDLP, DDLP, SDFP, DDFP, ODLP and ODFP procured change in color shown in below table 1. The presence of phytochemical is shown in table 2. The aqua extract does not showed much effect on phytochemicals but when the extract was prepared separately stickiness was observed in case of fruits which were explored in sunlight than in other solvents. The TLC and UV of each extract offered indepth knowledge of behavior pattern of phytochemicals. The alkaloids are nitrogen containing compound change it strength toward red shift. White flavonoids have changed its colour to buff it may be due modification of group. Saponin was absent in methanol extract of dark while it is present water extract in all conditions similarly tannin was nor shown in sunlight explored water extract of leaves. Flavonoid may change its ring structure in oven dried sample. The all sample exhibited similar rf values in all extract and all condition. The result indicates that there is no change in behavior of *Ageal marmaloes* phytochemicals in other conditions. UV of leaves extracted in methanol showed λ_{max} value 332 nm and in

ethanol showed λ_{max} 274 nm while fruits exhibited λ_{max} 222 nm & 219 nm respectively. Phytochemicals from medicinal plants served as lead compounds for drug discovery and design. *Ageal marmaloes* have both polar and non-polar phytoconstituents, therefore aqua and organic solvents may be considered as a suitable solvent for further pharmacological research.

Tables 1: Ageal marmaloes Phytochemicals represented change in colour in leaves and fruits extract

Phytochemicals	Change in colour
Reducing sugar	Brick red precipitate
Tannin	brown color
Phlobatannins	Deposition of red precipitation
Saponins	froth formation
terpenoids	reddish brown interface
Alkaloids	yellow colour
Flavonoids	red color
Polyphenols	Colorless to brown

Table 2: The presence of phytochemicals from *Ageal marmaloes* leaves and fruits in dark , light and oven dried powder extracted in solvent and aqua conditions (* + present – absent)

Phytochemicals	Phytochemicals in Dark Conditions						Phytochemicals in Light						Phytochemicals in oven dried sample					
	water		methanol		Ethanol		Water		Methanol		Ethanol		water		methanol		Ethanol	
	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves
Reducing sugar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannin	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Phlobatannins	-	+	-	-	+	-	-	+	+	+	+	+	-	-	-	-	-	-
Saponins	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+
terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
polyphenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3 : Rf values of *Ageal marmaloes* SDLP, DDLP, SDFP, DDFP, ODLP and ODFP

Solvents	Phytochemicals in Dark condition		Phytochemicals in Sunlight condition		Phytochemicals in Oven dried condition	
	Fruit	leaves	Fruit	leaves	Fruit	leaves
Methanol	0.96	0.05,0.25,0.80,0.90	0.92	0.05,0.25,0.80,0.90	0.92	0.05,0.25,0.79,0.90
Ethanol	0.90	0.05,0.25,0.79,0.90	0.96	0.05,0.25,0.79,0.90	0.92	0.05,0.25,0.79,0.90
Water	0.89	0.05,0.25,0.80,0.90	0.92	0.05,0.25,0.80,0.90	0.92	0.05,0.25,0.79,0.90

Conclusion:

The presence of phytochemical/bioactive compounds like tannin, phenols, flavonoids, alkaloids and steroids in leaves & fruits of *Aegle marmelos* supports the traditional use of this leaves & fruit by the rural population of India. There is no significant change in behaviour was observed in Dark, Sunlight and oven dried extract of solvents and in aqua condition.

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