



**DETERMINATION OF PHYTOCONSTITUENTS, CHROMATOGRAPHY
STUDY OF *AEGLE MARMELLOS* BY USING VARIOUS EXTRACT**

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ABSTRACT

Aegle marmelos various extract was used for investigation of phytoconstituents and chromatography. Different extract have posse wide range of active constituents. Investigation confirmed by using synthetic antioxidant. In addition, the separation and purification of the compounds, column chromatography, was performed. Column chromatography means of a gradient solvent system

INTRODUCTION:

Medicinal plants are important for treating various diseases. Medicinals have higher number of phytoconstituents which is used valuable for drug discovery. Ancient searches or record i.e. Charak Samhita, Rigveda, Yajurveda, Atharveda and Sushrit Samhita detailed explanation for various medicinal plants and its treatment [1]. Last decades drug discovery has been increased and advanced scientific technology used for ruleout various pharmacological properties of medicinal plants i.e. antibacterial activity, antifungal activity, anticancer activity, antioxidant activity,

hepatoprotective activity, anti-inflammatory activity and larvicidal activity [2-12].

The bilva tree itself is so holy and fortunate that its reverence or its implication is mention in many Puranas and other scriptures at diverse instance. Born from the breasts of Goddess Lakshmi, the Bilva tree is ever dear to Mahadeva. So I ask this tree to offer a Bilva leaf to Lord Shiva. This has various effects. One of them is the reduction of raja-tama particles present in the atmosphere. A Sattvik leaf like bilva

patra when brought in proximity of a person suffering from negative energy, distress than the black energy present within him is reduced [13].

MATERIAL AND METHOD

Bael. was collected from baron district of Rajasthan. These were authenticated by botanist. These plants were preserved for further reference.

Preparation of plant extract: Bael leaves were sun dried for 8 days. After these help of grinder these were grinded and prepared different extract of these powder.

Phytochemical screening

Phytochemical examinations were carried out extracts as per the following standard methods.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formations of yellow colour precipitate indicate the occurrence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Tannins

a) Gelatin test

To 1 ml of the plant extract was added few drops of 1% Gelatin solution containing 10% Sodium chloride (NaCl). Formation of

white precipitate indicates the presence of Tannins.

9. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald, green colour indicate the presence of diterpenes [14-15].

1.5 Thin layer chromatography

Thin layer chromatography is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for

TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) for Quercetin and toluene: ethyl acetate: formic acid (7:5:1) for gallic acid solvent system used (Patel *et al.*, 2017; Sajeeth *et al.*, 2010). After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f), values were calculated for different samples [16-19].

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

2 Result of Phytochemical Screening

Table 1: Result of phytochemical screening of leaves extract of *Aegle marmelos*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids Wagner's Test:	-ve	-ve	-ve	-ve
2.	Glycosides Legal's Test:	-ve	-ve	-ve	-ve
3.	Flavonoids Alkaline Reagent Test: Lead acetate Test:	+ve +ve	-ve +ve	-ve +ve	+ve +ve
4.	Diterpenes Copper acetate Test:	+ve	+ve	+ve	+ve
5.	Phenol Ferric Chloride Test:	-ve	-ve	+ve	+ve
6.	Proteins Xanthoproteic Test:	-ve	-ve	+ve	+ve
7.	Carbohydrate Fehling's Test:	-ve	-ve	+ve	-ve
8.	Saponins Froth Test:	-ve	+ve	+ve	+ve
9.	Tannins Gelatin test:	-ve	-ve	-ve	-ve

2. Results of Thin layer chromatography

Table 2: Calculation of Rf Value of leaves extract of *Aegle marmelos*

Identification of flavonoids			
S. N.	Mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1)	Spot Distance	Rf value
1.	(Quercetin) Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV=1 No. of spot at normal light=1	Long – 3.5 Short – 3.5 Normal light – 3.5	Long – 0.7 Short – 0.7 Normal light – 0.7
2.	(Chloroform extract) Dis. Travelled by mobile phase= 4.5cm No. of spot at long UV= 13 No. of spot at short UV=9 No. of spot at normal light =8	Long – 1.2, 1.9, 2.2, 2.5, 2.7, 2.9, 3.3, 3.5, 3.7, 3.9, 4.1, 4.3, 4.6 Short – 2.5, 2.7, 3.0, 3.2, 3.4, 3.7, 3.9, 4.2, 4.6 Normal light – 2.5, 3.3, 3.5, 3.9, 4.0, 4.3, 4.5, 4.7	Long – 0.24, 0.38, 0.44, 0.5, 0.54, 0.58, 0.66, 0.7, 0.74, 0.78, 0.82, 0.86, 0.92 Short – 0.5, 0.54, 0.6, 0.64, 0.68, 0.74, 0.78, 0.84, 0.92 Normal light – 0.5, 0.66, 0.7, 0.78, 0.8, 0.86, 0.9, 0.94
3.	(Ethyl acetate extract) Dis. Travelled by mobile phase= 4.5cm No. of spot at long UV= 10 No. of spot at short UV=7 No. of spot at normal light =7	Long – 1.6, 2.1, 2.6, 3.0, 3.4, 3.7, 4.0, 4.2, 4.4, 4.7 Short – 2.6, 3.0, 3.3, 3.6, 3.9, 4.2, 4.4 Normal light – 3.1, 3.4, 3.6, 4.0, 4.2, 4.4, 4.5, 4.8	Long – 0.32, 0.42, 0.52, 0.6, 0.68, 0.74, 0.8, 0.84, 0.88, 0.94 Short – 0.52, 0.6, 0.66, 0.72, 0.78, 0.84, 0.88 Normal light – 0.62, 0.68, 0.74, 0.8, 0.88, 0.92, 0.96
4.	(Ethanol extract) Dis. Travelled by mobile phase= 4.5cm No. of spot at long UV= 8 No. of spot at short UV=9 No. of spot at normal light =8	Long – 1.6, 2.7, 3.0, 3.3, 3.6, 3.9, 4.2, 4.6 Short – 2.7, 3.0, 3.2, 3.4, 3.6, 3.9, 4.2, 4.5, 4.7 Normal light – 2.9, 3.3, 3.6, 4.0, 4.2, 4.4, 4.5, 4.8	Long – 0.32, 0.54, 0.6, 0.66, 0.72, 0.78, 0.84, 0.92 Short – 0.54, 0.6, 0.64, 0.68, 0.72, 0.78, 0.84, 0.9, 0.94 Normal light – 0.6, 0.64, 0.97
5.	(Aqueous extract) Dis. Travelled by mobile phase= 4.5cm No. of spot at long UV= 5 No. of spot at short UV=2 No. of spot at normal light =1	Long – 2.5, 3.4, 3.6, 4.3, 4.6 Short – 3.6, 4.8 Normal light – 4.8	Long – 0.5, 0.68, 0.72, 0.86, 0.92 Short – 0.7, 0.96 Normal light – 0.96

Identification of phenol			
S. N.	Mobile phase Toluene: Ethyl acetate: Formic acid (7:5:1)	Spot Distance	Rf value
1.	(Quercetin) Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV=1 No. of spot at normal light =1	Long – 2.3 Short – 2.3 Normal light – 2.3	Long – 0.46 Short – 0.46 Normal light – 0.46
1.	(Methanol extract) Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 11 No. of spot at short UV=9 No. of spot at normal light =7	Long – 0.6, 1.3, 2.0, 2.3, 2.6, 2.9, 3.3, 3.5, 3.7, 4.2, 4.6 Short – 1.8, 2.3, 2.6, 3.0, 3.4, 3.8, 4.2, 4.6, 4.8 Normal light – 2.4, 3.0, 3.4, 3.7, 4.2, 4.6, 4.8	Long – 0.12, 0.26, 0.4, 0.46, 0.52, 0.58, 0.66, 0.7, 0.74, 0.84, 0.92 Short – 0.36, 0.46, 0.52, 0.6, 0.68, 0.76, 0.84, 0.92, 0.96 Normal light – 0.48, 0.6, 0.68, 0.74, 0.84, 0.92, 0.96
2.	(Aqueous extract) Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 3 No. of spot at short UV=0 No. of spot at normal light =0	Long – 2.3, 3.1, 4.8 Short – 0 Normal light – 0	Long – 0.46, 0.62, 0.96 Short – 0 Normal light – 0

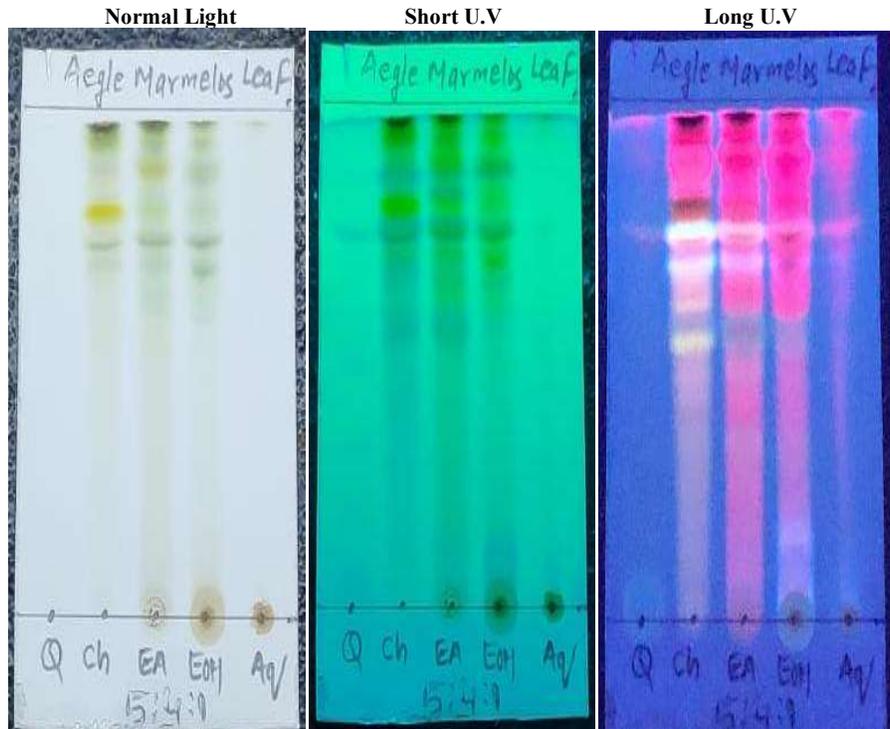


Figure 1:
Spot-1= Quercetin, Spot-2= Leaves extract of *Aegle marmelos*

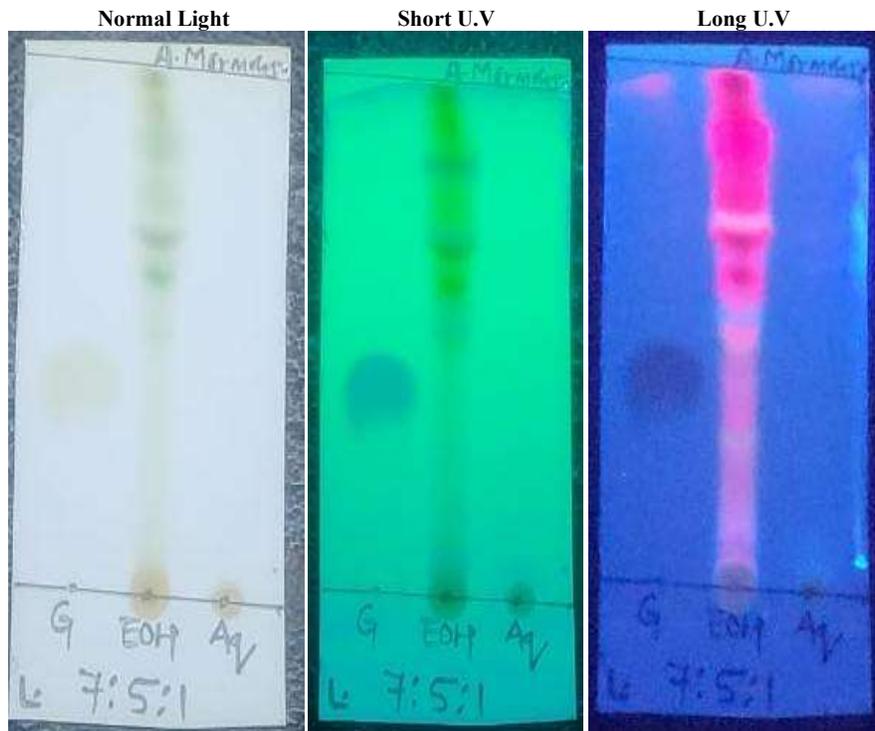


Figure 2
Spot-1= Gallic acid, Spot-2= Leaves extract of *Aegle marmelos*

CONCLUSION:

This investigation confirmed by using synthetic antioxidant. In addition, the separation and purification of the compounds, column chromatography, was performed. Column chromatography means of a gradient solvent system. The column was subjected to elution using a gradient solvent system. The fractions were analyzed for their antioxidant activities by DPPH, SOD, and FRAP assays. Moreover, among all the fractions isolated through column significant ferric reducing ability, DPPH, and superoxide scavenging activity.

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