

**A STUDY ON IDENTIFICATION, SEPARATION AND
CHARACTERIZATION OF THIO PHENOL FROM *AEGLE
MARMELOS***

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ABSTRACT

Right from the beginning, the Siddha Medicines obliterate the microbes and paves a way for hale and healthy life to the primitive stage of the mankind. Astonishingly, the plant *Aegle marmelos* poises a sharper reaction while using it as antioxidant activity. The interaction with the other compounds ventures into the new researches. The mysteries behind its interactions are not fully divulged and only the nature itself could reveal.

KEYWORD: *Aegle marmelos*, antioxidant activity.

1. INTRODUCTION

Herbal medicine also called botanical medicine or phytomedicine - refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease. Herbal medicine is the science of using herbal remedies to treat the sick. It is therefore covers everything from medicinal plants with powerful actions, such as Digitalis and Belladonna, to those with very gentle action, such as chamomile, mint and many others. Medicinal plants which form the backbone of traditional medicines have in the last few decades been the subject for very intense pharmacological studies. These medicinal plants are the potential source of new compounds of therapeutics value and as sources of lead compounds in the drug development. In the developing

countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care. There arises a need therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies (Gurav, S, *et al.*, 2007).

Thiophenol

Thiophenol is an organosulfur compound with the formula C_6H_5S and sometimes abbreviated as PhSH. This foul-smelling colorless liquid is the simplest aromatic thiol. The chemical structures of thiophenols are analogous to phenols except the oxygen atom in the hydroxyl group (-OH) bonded to the aromatic ring is replaced by a sulfur atom. The prefix **thio-** implies a sulfur-containing compound and when used before a root word name for a compound which would normally contain an oxygen atom, in the case of 'thiol' that the alcohol oxygen atom is replaced by a sulfur atom. Thiophenols also describes a class of compounds formally derived from thiophenol itself. All have a sulfhydryl group (-SH) covalently bonded to an aromatic ring. The organosulfur ligand in the medicine thiomersal is a thiophenol. Using thiophenol as a reactant.

Aegle marmelos (L.) Corr., (Rutaceae) is a popular medicinal plant in the Ayurvedic and Siddha systems of medicine and folk medicines used to treat a wide variety of ailments. The plant, popularly known as the bael tree, is native to the Indo-Malayan region (Hooker JD, 1975) and is currently cultivated in India, Pakistan, Bangladesh, Sri Lanka, Burma, and Thailand (Islam R, *et al.*, 1995). The tree is a slender, aromatic perennial, 6.0–7.5 m tall and 90–120 cm in girth. It flowers from May to July and yields an annual average of 300–400 fruits (200–250 kg) per tree. Various parts of the tree, including the fruit, possess medicinal properties. The roots are useful for treating diarrhoea, dysentery, and dyspepsia (Mazumder R, *et al.*, 2006). The leaf is used for ophthalmia, diabetes, and asthmatic complaints. Unripe fruit is useful for treating diarrhoea, dysentery and stomachalgia. The aqueous extracts of the stem and root bark are used to treat malaria, fever, jaundice, and skin diseases such as ulcers, urticaria, and eczema (Nadkarni K, *et al.*, 1954). In pharmacological trials, both the fruit and root showed antiamoebic and hypoglycaemic activities (Ponnachan PTC, *et al.*, 1993., Kamalakkannan N, *et al.*, 2005). The plant is rich in alkaloids, among which aegline, marmesin, marmin, and marmelosin are the major ones. Aqueous leaf extract and methanolic extract of the root bark of *A. marmelos* showed preventive effects on myocardial diseases (Kakiuchi N, *et al.*, 1991., Prince PS, *et al.*, 2005). The compounds luvangetin and pyranocoumarin, isolated from the seeds of *A. marmelos*, showed significant antiulcer

activity (Goel R.K, *et al.*, 1997). Essential oil isolated from the leaf has antifungal activity (Rana B.K, *et al.*, 1997). The aqueous extract of leaf possesses a hypoglycaemic effect (Das A.V, *et al.*, 1996).

2. MATERIAL AND METHODS

2.1 Plant materials

The matured *Aegle marmelos* were collected from Puliyakkudi, Thiruvarur District, Tamil Nadu, India in July 2015. The *Aegle marmelos* were identified (Am 003) and authenticated by Botanist, Dr.M.Jagadeesan, Department of environmental and Herbal medicine, Tamil University, Thanjavur.

2.2 Preparation of Plant Extracts

The plant material was crushed using kitchen blender and that powder was subjected to cold extraction with ethanol for 24 hrs. The solvent extract was distilled and condensed at 40°C. The condensed extract was stored at room temperature in air tight bottles and used for further studies.

2.3 Isolation of phenol by Column Chromatography

The bottom of the column was first plugged with little glass wool and then clean sand bed was placed over the glass wool. The sand bed serves to give a flat base to the column of the adsorbent. Then the dried Silica Gel 100-200 mesh was poured into the column. After 2/3rd of the column was filled with the powder, it was tamped, and set aside. After that, a filter paper disc and sand bed were placed over the adsorbent in order to avoid the disturbance of the adsorbent, as fresh mobile phase was added to the column in the initial stages of development. The ethyl acetate (9%) hexane (1%) extract of sample was placed over the filter paper disk and used to isolate the active constituents. The phenol was subjected to column chromatography over silica gel 100-200 mesh. The column was eluted with solvents of increasing order of polarity. The fractions were collected in 25ml each and allowed to evaporate to get the residue.

2.4 Thin layer chromatography

Thin layer chromatography is one of the valuable and versatile methods for analysis of wide range biomolecules. TLC is nothing but a modification of paper chromatography where the sheet of paper is replaced by thin layer of absorbent material. Therefore the separation in

TLC is also due to the differential partition of solutes between the stationary and mobile phases.

2.4.1. Principle

The general principle involved in tlc is similar to that of column chromatography, i.e., adsorption chromatography. In the adsorption process the solute competes with the solvent for the surface sites of adsorbent. Depending on the distribution coefficients the compounds are distributed on the surface of the adsorbent of course, in tlc the partition effect in the separation is also not ruled out the adsorbent normally used contains a binding agent such as calcium sulphate which facilitates the holding of the adsorbent to the glass plate.

2.4.2. Procedure

The stationary phase is prepared as slurry with water or buffer at 1:2 and applied to a glass plate or an inert plastic or aluminium sheet, as thin uniform layer by means of a spreader such as glass rod or pipette or using a tlc applicator. (0.25 mm thickness for analytical separations and 2 – 5 mm thickness for preparative separations as prepared)

2.4.3. TLC Plate Preparation

The solid phase of silica gel was kept in hot air oven in 100°C for 20 minutes. Then the silica powder was mixed with ethanol and the slurry was prepared. The 12 x 6 cm clean TLC glass plates were taken and it was covered with that slurry and allowed to air dry. After drying the plates were kept in hot air oven in 100°C for 8 hours. After developing the plates the condensed filtrate was spotted using capillary tube. The different spots were separated using a different solvent mixture act as mobile phase it was given below.

2.4.4. Sample Application

Draw a line lightly with a pencil about 1.5 – 2.0 cm from the bottom. If the thin layer is too soft to draw a pencil line, place a scale at the bottom and spot at a distance of 1.5 cm. Note down the order. The samples are spotted using capillary tube at 1.5 cm distance between them. For preparative TLC, the sample is applied as a band across the layer rather than as a spot.

2.4.5. Plate Development

The chromatographic tank is filled with developing solvent to a depth of 1.5 cm and equilibrated for about 5 hrs. The thin layer plate is placed gently in the tank and allowed to stand for about 60 mins. Make sure the spots do not touch the solvent directly. Capillary action

caused the solvent to ascend as in paper chromatography and the separation of compounds takes place. As the solvent front reaches about 1-2 cm from the top of the plate, the plate is removed, solvent front is marked with a pencil immediately and allowed to air dry placing the plate upside down.

2.4.6. Isolation of Phenolic Compound

The condensed filtrate was used for chromatography. The benzene compound was separated using ethyl acetate and hexane solvent mixture in the ratio of 9:1. The R_f values of these compound were recorded under visible light.

2.4.7. Characterization of phenolic compound by FT-IR

The presence of phenolic compound in the *Aegle marmelos* was studied by Fourier Transform Infrared (FT-IR) spectroscopy. A FT-IR spectrometer was used to record IR spectra. A potassium bromide micro disk was prepared from finely identified compound of 2 mg of sample with 100 mg of K Br.

3. RESULTS AND DISCUSSION

In this present study thiophenol was identified and isolated after initial screening. The characterization of bio active compound has also been done using UV-FTIR. Using column chromatography the compound thiophenol was purified from the extract of *Aegle marmelos* and it was subjected to kept for further analysis. The results indicate the presence the purified thiophenol and the results are depicted in fig: 1. Alkaloids are important defence of the plant against pathogenic organism and herbivores. It also toxin for insects which further modify the alkaloids and incorporate them into their own defence secretion (Khanuja, 2002).

Thin Layer Chromatography

The TLC profile of secondary metabolites thiophenol is tabulated in the table-1. Among the six groups of phytochemical constituents in the sample of *Aegle marmelos*, the thiophenol, was found to be the most abundant one followed by phenol.

The Chromatography showed that of the extract of *Aegle marmelos* positively reacts to the presence of phenol. The presence of some of these compounds had been demonstrated previously by other researchers. However some of the results obtained are not in agreement with the previous findings. This might be due to climatic and environmental factors.

Table: 1- Thin layer Chromatography

COMPOUND	R _f VALUE
Thio phenol	0.44

Spectrum Analysis of Thiophenol

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum profile was illustrated in the Figure 2. The FTIR spectrum confirmed the presence of phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds. Hence, the compound subjected to UV-VIS and FTIR analysis is used for the identification of thiophenol present in *A. marmelos*. In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition. The FT-IR spectrum of compound contains seven peaks (Fig. 2). Of that, five peaks are characteristics of 3454.26 cm⁻¹(polymeric OH stretch), 1600.01 cm⁻¹(C=O stretch), 1099.25 cm⁻¹(Phenol (or) tertiary alcohol, OH bend), 800.88 cm⁻¹(C-O stretch).

FTIR analysis of the SBL extracts shows a strong presence of hydroxyl group which is common in all phenolic compounds. All SBL extracts absorption bands were attributed to (OH) stretching vibrations from phenols, a group of compounds (chemical) containing hydroxyl functional groups (-OH) attached to an aromatic hydrocarbon. Phenolic compounds from natural resources displayed antifungal activity (Soundararajan *et al.*, 2012). Many active compounds were produced by plants which contained these active groups (secondary metabolites). Certainly, other chemical components of the extracts could also contribute, although lack of chemical profiling has never been reported on this. It is possible that these compounds are mainly responsible for the Antioxidant activities observed in this study. In line with the findings of various authors the fact that *Aegle marmelos* possesses medicinal property in terms of Anti oxidant, Anti cancer Cardio protective, Anti hyperglycemic and Anti inflammatory is clearly revealed by the present study. The phytochemical screening of the crude extract of *A.marmelos* revealed the presence of Alkaloids, Cardiac glycosides, Terpenoids, Saponins, Tannis, Flavonoids, and Steroids (Venkatesan, *et al* ., 2009). Manjeshwar Shrinath Baliga *et al.*, 2011 indicated that the *Aegle marmelos* fruit possesses broad range of therapeutic effects that includes free radical scavenging, antioxidant, inhibition of lipid peroxidation, antibacterial, antiviral, anti-diarrheal, gastroprotective, anti-ulcerative colitis, hepatoprotective, anti-diabetic, cardio protective and radio protective effects.

The plant *Aegle marmelos*, is having great potential to cure the disease like diabetes, cholesterol, peptic ulcer, inflammation, diarrhoea, and dysentery, anticancer, cardio protective, anti bacterial, anti fungal, radio protective, anti pyretic, analgesic, constipation, respiratory infection, antioxidant, hepatoprotective, wound healing and many more. The present review summarizes the scientific information of various aspects of *Aegle marmelos* plant used in traditional system of medicine for variety of purpose (Pushpendra K. Patel, *et al.*, 2012).

Fig: 1 UV analysis of this phenol

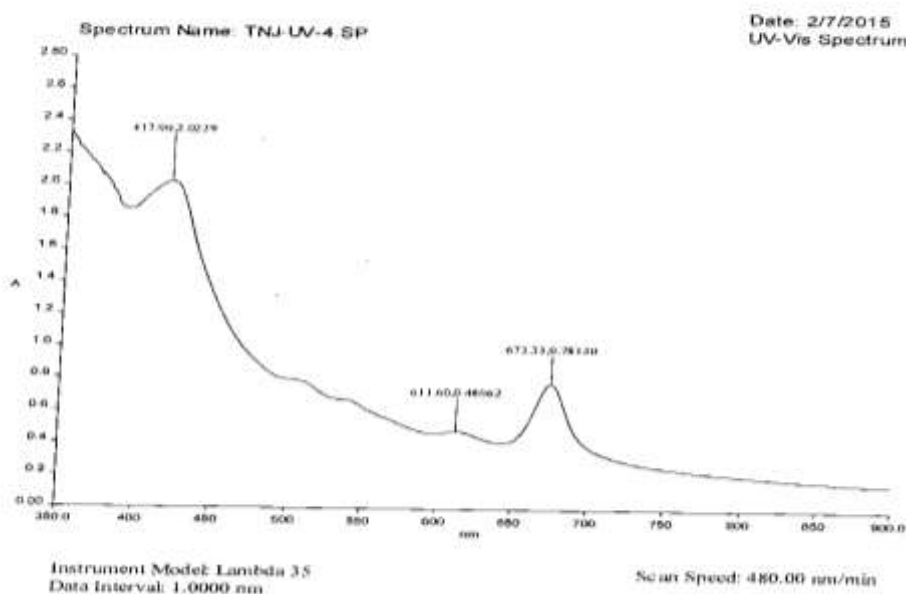
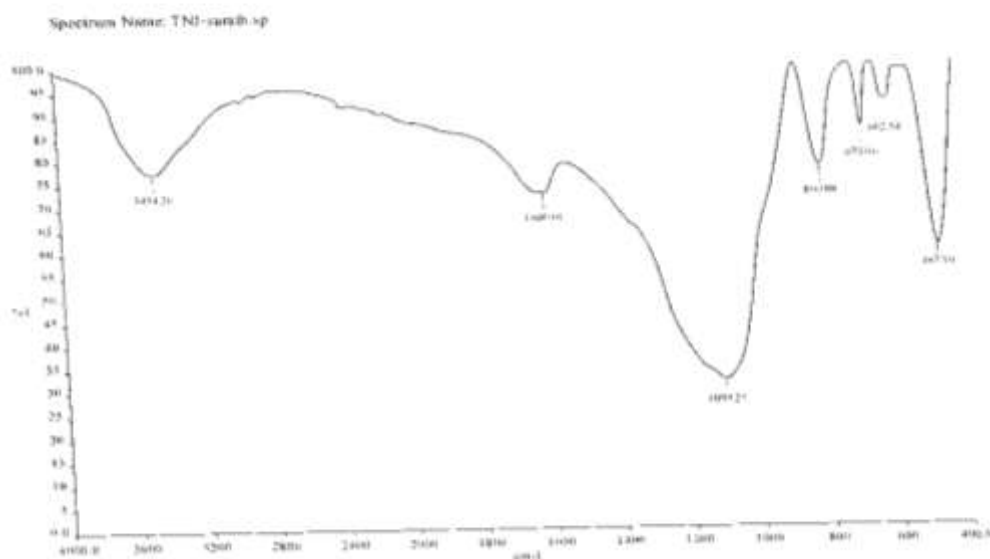


Fig: 2 FT-IR analysis of this phenol



4. SUMMARY AND CONCLUSION

The existence of phenolic compound is found out while subjecting to the phytochemical screening. In this, the ethanol is being used to extract plant *Aegle marmelos*. The presence of thiophenol confirmed during the course of column chromatography and thin layer chromatography.

These phenolic compounds resist the growth of fungus ie antifungal maintaining the natural imbalance. The therapeutic agent serves to annihilate the microbes-medicinal purposes. In FTIR techniques the omnipotent of hydroxyl group being the driving force behind the entire activity which is being normally in all phenolic compounds. It is obvious that the stretched vibration from phenols corroborated to the thiophenol absorption bands which are meant to be a strong bond between the hydroxyl functional groups and the aromatic hydrocarbon. In this field of pharmacology this separated thiophenol compounds chemical properties do sharper reactions-an eye opener to the several medicinal researches.

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