INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

# EVALUATION OF PHYTOCONSTITUENTS IN AQUEOUS AND ORGANIC EXTRACTS OF AEGLE MARMELOS (L.) CORREA. AND PUNICA GRANATUM L.

**I. Mariya Ramya, S. Rekha and A. Parvathi***

Herbal Study Centre, Department of Botany, Holy Cross College (Autonomous) Tiruchirappalli - 620 002, Tamil Nadu, India.

**ABSTRACT**

The present study was carried out to evaluate various therapeutic phytochemicals in different organic(ethanol, chloroform, dimethyl sulfoxide (DMSO), methanol) and aqueous extracts of leaves and fruits (dried fruit rind) of *Aegle marmelos* (L.) Correa.and *Punica granatum* L. The different parts of these two plants are extensively used in Indian traditional systems of medicine to cure chronic diarrhoea, dysentery, inflammations, indigestion and acute bronchitis. Qualitative and quantitative phytochemical analyses of these plant parts have revealed the possession of active phytochemical constituents such as alkaloids, cardiac glycosides, flavonoids, indoles, phenols, saponins, steroids, tannins and terpenoids which have tremendous therapeutic and pharmaceutical potential.

**Keywords:** *Aegle marmelos, Punica granatum,* qualitative analysis, quantitative estimation.

## INTRODUCTION

From time immemorial, people of India have been using thousands of medicinal plants for curing various diseases and about 8000 herbal remedies have been codified in Ayurveda. Since ancient times, the medicinal plants and their parts like leaves, flowers, fruits, seeds, bark and heartwood have been used as an excellent source in treating various diseases as they possess active constituents that are used in the treatment of many human diseases. They are processed in the form of paste, juice, infusion, teas, powders, decoctions, extracts, syrups, pills, tablets, oils, medicated wines etc., before administration to a patient [1-3]. The acceptance of traditional medicine as an alternative form of health care has lead various authors to investigate the antimicrobial potential and phytochemical constituents of several medicinal plants[4-8]. Due to ever increasing need and cost of drugs especially in developing countries, a need to search for low cost drugs from natural sources becomes imperative.Angiospermic flora are the rich source of effective chemotherpeutants. Tiruipura Sundari *et al*., (2012)[9] in their study on phytochemical analysis of some therapeutic medicinal flowers recorded several therapeutic and pharmaceutically important secondary metabolites. Gnana Sundari *et al*., (2013)[10] in their report on phytochemical evaluvation of three species of *Solanum* L. registered the presence of high amount of therapeutic phytochemicals in different parts of the selected species. Hence, the present study was focused on evaluation of various phytoconstituents of aqueous and organic extracts of *Aegle marmelos* and *Punica granatum* that are used in Indian traditional systems of medicine like Ayurveda, Siddha and folk medicine to cure chronic asthma, diarrhoea, dysentery, intestinal disorders and dermatological infections.

*Aegle marmelos* (L.) Correa is commonly known as bael, belongs to the family Rutaceae, is a moderate sized, slender and aromatic tree. In Tamil, it is called vilvam indigenous to India. It is a deciduous sacred tree associated with Lord Shiva. Bael is reported to contain a number of coumarins, alkaloids, sterols and essential oils[11]. A drug balae fructose extracted from the fruits with mucilage and pectin content is very useful for treating chronic diarrhoea, dysentery, hemorrhoids and swellings. The leaf juice mixed with black pepper is used to treat jaundice and

asthmatic complaints. The antibiotic potential of the leaf, fruit and root helps in curing diarrhoea, dysentery, asthma and fever. The bark and root soaked in water for a over night in a copper vessel are used to cure blood pressure, diabetes and leprosy[12-14] .

*Punica granatum* L., is commonly known as pomegranate belongs to the family Punicaceae. In Tamil, it is called madhulam. It is a small tree with several upright thorny stems and elliptic leaves. The leaves, flowers and rind of the fruit are reported to be used in folklore medicine to treat gastro intestinal problems, diarrhoea, blood purification, leprosy, bronchitis and hypertension[15].

The plant is reported to contain over 28% of gallo tannicacid and the alkaloids pelletieri ne, methylpelletierine, isopelletierine, psuedopelletierine,gallic acid, tannic acid, sugar, calcium oxalate etc[16]. The present study was aimed at analysing the potential phytochemical constituents of the selected two ancient medicinal plants.

## MATERIALS AND METHODS

The leaves and fruits (dried fruit rind) used in the present study were collected from Sobanapuram, the foothills of the Pacchaimalais, Tiruchirappalli District, Tamil Nadu. The voucher specimens (404, 405) have been deposited at Herbal Study Centre, Department of Botany, Holy Cross College (Autonomous) Tiruchirappalli. The plants were taxonomically identified with the aid of "Flora of the Presidency of Madras" [17] and "Flora of Tamil Nadu Carnatic"[18].

The collected plant parts were cleaned, shade dried and powdered in an electric grinder. Fifteen grams of powdered plant materials soaked in 100 ml. of distilled water and 80% of ethanol, dimethyl sulfoxide, chloroform and methanol solvents were incubated for 24 hours. They were filtered using standard Whatman No.1 filter paper and the filtrate was allowed to evaporate at low temperature 10°C using Buchi Rotavapor. The extracts were stored in refrigerator and used for further analysis.

### Qualitative detection of Phytochemicals

Qualitative phytochemical analysis was carried out with different organic extracts of leaves and fruits (dried fruit rind) of the selected plants for identification of their bioactive components like alkaloids, cardiac glycosides, flavonoids, indoles, phenols, polyphenolases, proteins, saponins, steroids, tannins and triterpenoids using standared procedures[19-21]. The extracts were treated with various chemical reagents and their colour reactions were observed to identify the presence of different phyto-constituents.

### Quantitative estimation of phytochemicals

After the confirmation of the presence of various phytoconstituents by qualitative phytochemical analysis, the coarse powder of the plant material was taken up for the quantitative estimation of amino acids, flavonoids, phenols, proteins, reducing sugars, tannins and total sugars .

### Determination of total protein content

Fifty milligrams of sample was homogenized with 5 ml of ice-cold phosphate buffer and centrifuged at 2000 rpm for 5 minutes. To the supernatant solution, equal volume of 10% ice-cold TCA was added and incubated for 10 min. at 4°C for an hour. The precipitated protein was centrifuged and the pellet was dissolved in one ml of 0.1N NaoH. 0.5 ml of the protein solution was mixed with 5 ml of alkaline copper reagent. It was shaken well and allowed to stand at room temperature for 10 min. Then, 0.5 ml of folin – ciocalteau reagent was added and the volume was made upto a known quantity using distilled water. Blank was prepared without the sample extract. After 30 minutes the optical density of the solution was read at 660 nm in Spectronic – 20 D[22].

### Determination of total free amino acid

Hundred milligram of dried sample powder was homogenized with 5 ml of 80 % ethanol and centrifuged at 2000 rpm for 10 min. The pellet was re-extracted with the same solvent and centrifuged again. The supernatant were pooled. To the supernatant equal volume of petroleum ether was added to remove the chlorophyll pigments using separation funnel. The lower layer was taken as sample. 0.5 ml of acetate buffer was added to the 1 ml of alcoholic extract, followed by 1 ml of 1% ninhydrin. The reaction mixture was heated for 15 min. in a boiling water bath at 100ºC for colour development. It was then cooled and the volume was diluted to 10 ml with distilled water. For blank, 0.5 ml distilled water was taken and all the reagents were added and carried out as above. The colour intensity was measured at 570 nm[23].

### Determination of total carbohydrates

Hundred milligram of dried sample powder was homogenized with 5 ml of 80 % ethanol and centrifuged at 2000 rpm for 10 min. The pellet was re-extracted with the same solvent and centrifuged again. The supernatant were pooled. To the supernatant, equal volume of petroleum ether was added to remove the chlorophyll

pigments using separation funnel. The lower layer was taken as sample. 1 ml of protein free carbohydrate solution was mixed with 3 ml of the anthrone reagent (0.2% in Conc.H2SO4). The reaction mixture was heated for 5 min. in a boiling water bath at 100ºC with the marble on the top of the test tube to prevent loss of water by evaporation. Suitable reagent blank was prepared. The colour intensity was measured at 620 nm in a Spectronic -20[24].

**Determination of total Flavonoids**

Two gram of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatmann filter paper No. 42(125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight[25].

**Determination of total phenols**

100 mg of the sample was extracted with 5 ml of 80% ethyl alcohol and centrifuged at 2000 rpm. The supernatant was taken for assay. One ml of folin–ciocalteau reagent was added to 0.5 ml of the alcoholic extract of the sample. 2 ml of 20 % sodium carbonate was added and heated for one min. After cooling, the solution was made up to 10 ml with distilled water. A blank was prepared by adding all the reagents except the sample. The absorbency was read at 650 nm in Spectrophotometer [24].

**Determination of reducing sugar**

100 mg of the sample was extracted with 3 ml of 80% ethyl alcohol and centrifuged at 2000 rpm. The supernatant was taken for assay. One ml of the sample, two ml of distilled water and one ml of alkaline copper tartarate reagent is added and boiled until the development of orange colour. Along with the sample, blank is also boiled (1ml of distilled water and 1 ml alkaline copper tartarate reagent). After boiling to the cooled tubes 1ml of arsenomolybdate reagent is added and made upto 10 ml using distilled water. The colour development is read 620 nm in a spectrophotometer[24].

**Determination of Tannin**

500 mg powdered sample material was transferred to 250 ml conical flask containing 75 ml of distilled water. The contents in the flask were boiled for 30 min, centrifuged for 2000 rpm for 20 min. The supernatant was collected in 100 ml volumetric flask and made up to a known volume. One ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml of distilled water. To this,5 ml of Folin-Denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100 ml. It was shaken well and left for 30 min. and the absorbance was read at 700 nm against a reagent blank water[26].

**RESULTS AND DISCUSSION**

Qualitative analysis of the different organic and aqueous extracts of the selected plants are presented in Table 1-5. The results of the ethanolic extracts represented in Table-1 showed the adequate presence of alkaloids, cardiac glycosides, flavonoids, indoles, phenols, saponins, steroids and terpenoids. However, the test for HCN was uniformly negative. The leaves of *Punica granatum* alone showed the presence of polyphenolases (Hot Water test). Except the fruit rind of *Aegle marmelos* all the plant parts showed the presence of tannins. Our results fall in line with the reports of Venkatesan *et al*., (2009)[12].

The results of methanolic extracts (Table-2) showed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids. However, the leaves of *Punica granatum* alone showed the absence of indoles. Whereas, presence of phenols were observed in all the tested plant parts except in fruit rind of *Aegle marmelos*. However, our results are in contrary to the reports of Thillai Sivakumar *et al*.,(2010)[27] where they reported the absence of alkaloids and steroids in fruit rind of *Punica granatum*.

The DMSO extracts (Table- 3) showed the presence of alkaloids, cardiac glycosides, flavonoids, indoles, phenols, saponins, steroids and terpenoids. Except the fruit rind of *Aegle marmelos* all the tested plant parts showed the presence of tannins in abundance.

The chloroform extract (Table-4) of selected plant parts showed the presence of cardiac glycosides, flavonoids, phenols, steroids and terpenoids. Alkaloids are present only in the leaves of *Aegle marmelos* and *Punica granatum*. Whereas, the leaves and fruit rind of *Aegle marmelos* contained indoles and saponins . Absence of tannins is observed in all the tested plant parts.

Aqueous extracts (Table-5) of all the parts of selected plants showed the presence of cardiac glycosides, flavonoids, phenols, steroids and terpenoids. Leaves and fruit rind of *Aegle marmelos* showed the presence of indoles and saponins . Whereas, the leaves of *Aegle marmelos* and *Punica granatum* alone contained the alkaloids. Absence of tannins observed in all the tested plant parts which is also reported by Hameed *et al*., (2011)[28].

The selected plant parts were quantified for metabolites such as amino acids, total sugars, reducing sugars, proteins, flavonoids, phenols and tannins. The results are presented in Tables 6 & 7and Figures 1 & 2. From the table -6, it is understood that the amino acid content of the fruit rind of *Aegle marmelos* was found to be highest (6.250 mg/g) followed by fruit rind of *Punica granatum* (4.2550 mg/g). The amino acid content was found to be in similar in the leaves of *Punica granatum* (3.813 mg/g) and *Aegle marmelos* (3.750 mg/g). High amount of total sugar was registered in the leaves (19.875 mg/g) and fruit rind (24.250 mg/g) of *Punica granatum* when compared to *Aegle marmelos*.

The fruit rind of *Punica granatum* (9.125 mg/g) contained high amount of reducing sugar when compared to *Aegle marmelos*. However, the low amount of reducing sugar is registered in leaves of *Punica granatum* (7.125 mg/g) and *Aegle marmelos* (4.275 mg/g). The protein content varied from 0.2 mg/g (leaves of *Punica granatum*) to 0.52 mg/g (fruit rind of *punica granatum*).

From table-7, it is understood that the fruit rind of *Aegle marmelos* contained high amount of flavonoids (216 mg/g) when compared to the fruit rind of *Punica grantum*. However, the leaves of *Punica granatum* contained 160 mg/g of flavonoid content when compared to the *Aegle marmelos* (104 mg/g). The phenol content was registered high in the leaves of *Punica granatum* (6.627 mg/g) followed by the fruit rind of *Aegle marmelos* (4.761 mg/g). However, low amount of phenol is recorded in leaves of *Aegle marmelos* (2.978 mg/g) and fruit rind of *Punica granatum* (0.1332 mg/g). The tannin content was high in the leaves of *Punica granatum* (43.668 mg/g) when compared to the leaves of *Aegle marmelos* (12.377 mg/g). However, maximum tannin content was registered in fruit rind of *Aegle marmelos* (12.377 mg/g) followed by *Punica granatum* (7.929 mg/g).

## CONCLUSION

This study indicated the presence of potential phytochemical constituents in leaves and fruit-rind of the *Punica granatum* and *Aegle marmelos*. The presence of high amount of tannins and flavonoids in these two plants supports the traditional and folkloric usage in treating chronic diarrhoea and inflammations. The presence of tannins contributes to the astringent potential and antidiarrhoeal nature of the plants. The non cyanophoric nature of these plant parts might supports the oral consumption without any hesitation. The positive response to the hot water test by the leaves of *Punica granatum* indicates the presence of polyphenolases that are antioxidant in nature.

## ACKNOWLEDGEMENTS

The authors are grateful to UGC, New Delhi for financial support and also thank Principal of Holy Cross College (Autonomous), Tiruchirappalli for providing research facilities.

**Table 1: Test with fresh and 80% ethanolic extracts**

| S.No | Name of the plants | Plant parts tested | AL | CG | FL | HCN | HW | IN | PH | SA | ST | TA | TE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Aegle marmelos*, (L.) Correa | Leaves | ++ | + | +++ | - | - | + | ++ | + | +++ | + | +++ |
| | | Fruit rind | + | + | +++ | - | * | + | ++ | + | +++ | - | +++ |
| 2. | *Punica granatum*, L. | Leaves | +++ | + | +++ | - | + | + | +++ | + | +++ | +++ | ++ |
| | | Fruit rind | ++ | + | +++ | - | * | + | +++ | + | +++ | +++ | + |

+++ = High intensive; ++ = Medium intensive; + = Low intensive; - = Negative; *= Not tested; AL=Alkaloids; CG = Cardiac glycosides; FL= Flavonoids; HW= Hot water; IN= Indoles; PH= Phenols; SA= saponins; ST= Steroids; TA= Tannins; TE= terpenoids

**Table 2: Test with 80% methanolic extracts**

| S.No | Name of the plants | Plant parts tested | AL | CG | FL | IN | PH | SA | ST | TA | TE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Aegle marmelos*, (L.) Correa | Leaves | + | + | +++ | + | + | + | +++ | + | ++ |
| | | Fruit rind | + | + | +++ | + | - | + | +++ | + | ++ |
| 2. | *Punica granatum*, L. | Leaves | + | + | +++ | - | +++ | + | + | + | + |
| | | Fruit rind | + | + | +++ | + | ++ | + | + | + | + |

+++ = High intensive; ++ = Medium intensive; + = Low intensive; - = Negative; *= Not tested; AL=Alkaloids; CG = Cardiac glycosides; FL= Flavonoids; HW= Hot water; IN= Indoles; PH= Phenols; SA= saponins; ST= Steroids; TA= Tannins; TE= terpenoids

**Table 3: Test with 80% Dimethyl sulfoxide (DMSO) extracts**

| S.No | Name of the plants | Plant parts tested | AL | CG | FL | IN | PH | SA | ST | TA | TE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Aegle marmelos*, (L.) Correa | Leaves | + | + | +++ | + | +++ | + | + | + | +++ |
| | | Fruit rind | + | + | +++ | +++ | +++ | + | +++ | - | +++ |
| 2. | *Punica granatum*, L. | Leaves | ++ | + | +++ | + | +++ | + | + | ++ | +++ |
| | | Fruit rind | ++ | + | +++ | ++ | +++ | + | +++ | ++ | + |

+++ = High intensive; ++ = Medium intensive; + = Low intensive; - = Negative; *= Not tested; AL=Alkaloids; CG = Cardiac glycosides; FL= Flavonoids; HW= Hot water; IN= Indoles; PH= Phenols; SA= saponins; ST= Steroids; TA= Tannins; TE= terpenoids.

**Table 4: Test with 80% Chloroform extracts**

| S.No | Name of the plants | Plant parts tested | AL | CG | FL | IN | PH | SA | ST | TA | TE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Aegle marmelos*, (L.) Correa | Leaves | + | + | +++ | + | + | + | +++ | - | + |
| | | Fruit rind | - | + | +++ | + | + | + | +++ | - | + |
| 2. | *Punica granatum*, L. | Leaves | + | + | +++ | - | + | - | + | - | + |
| | | Fruit rind | - | + | +++ | - | + | - | + | - | + |

+++ = High intensive; ++ = Medium intensive; + = Low intensive; - = Negative; *= Not tested; AL=Alkaloids; CG = Cardiac glycosides; FL= Flavonoids; HW= Hot water; IN= Indoles; PH= Phenols; SA= saponins; ST= Steroids; TA= Tannins; TE= terpenoids.

**Table 5: Test with aqueous extracts**

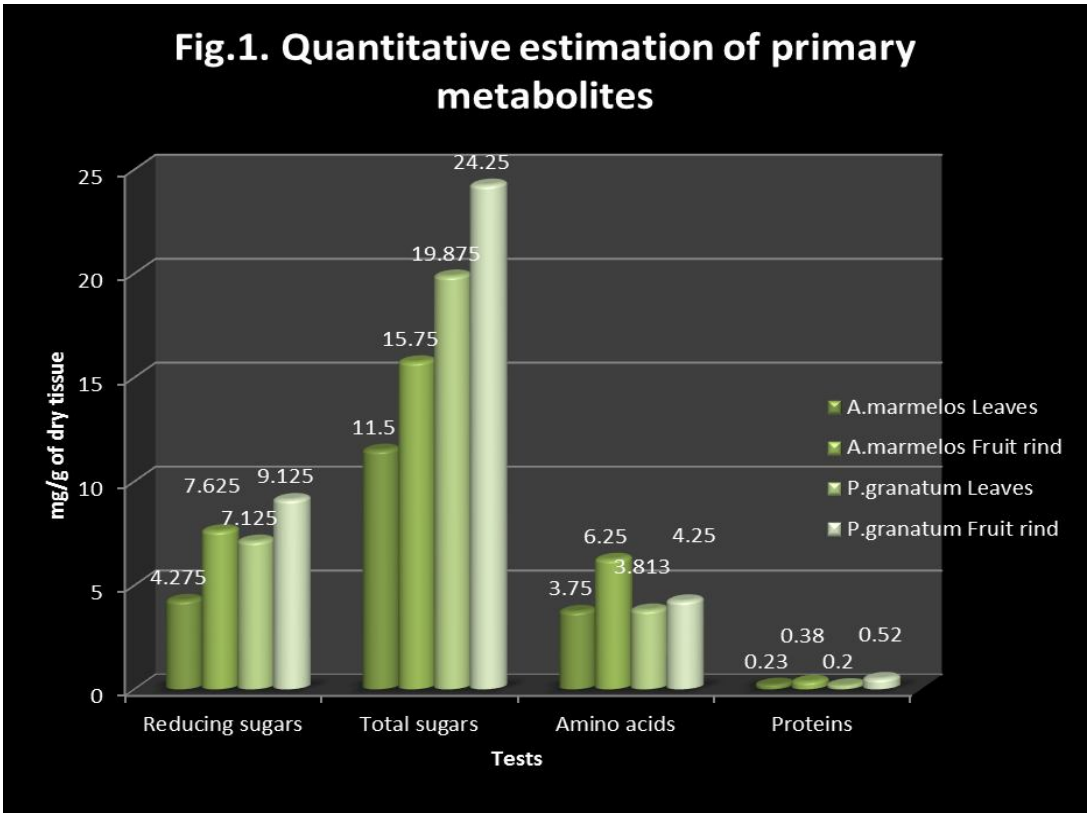
| S.No | Name of the plants | Plant parts tested | AL | CG | FL | IN | PH | SA | ST | TA | TE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | *Aegle marmelos*, (L.) Correa | Leaves | + | + | +++ | + | + | + | +++ | - | + |
| | | Fruit rind | - | + | +++ | + | + | + | +++ | - | + |
| 2. | *Punica granatum*, L. | Leaves | + | + | +++ | - | + | - | + | - | + |
| | | Fruit rind | - | + | +++ | - | + | - | + | - | + |

+++ = High intensive; ++ = Medium intensive; + = Low intensive; - = Negative; *= Not tested; AL=Alkaloids; CG = Cardiac glycosides; FL= Flavonoids; HW= Hot water; IN= Indoles; PH= Phenols; SA= saponins; ST= Steroids; TA= Tannins; TE= terpenoids

## QUANTITATIVE ANALYSIS

**Table 6: Estimation of primary metabolites**

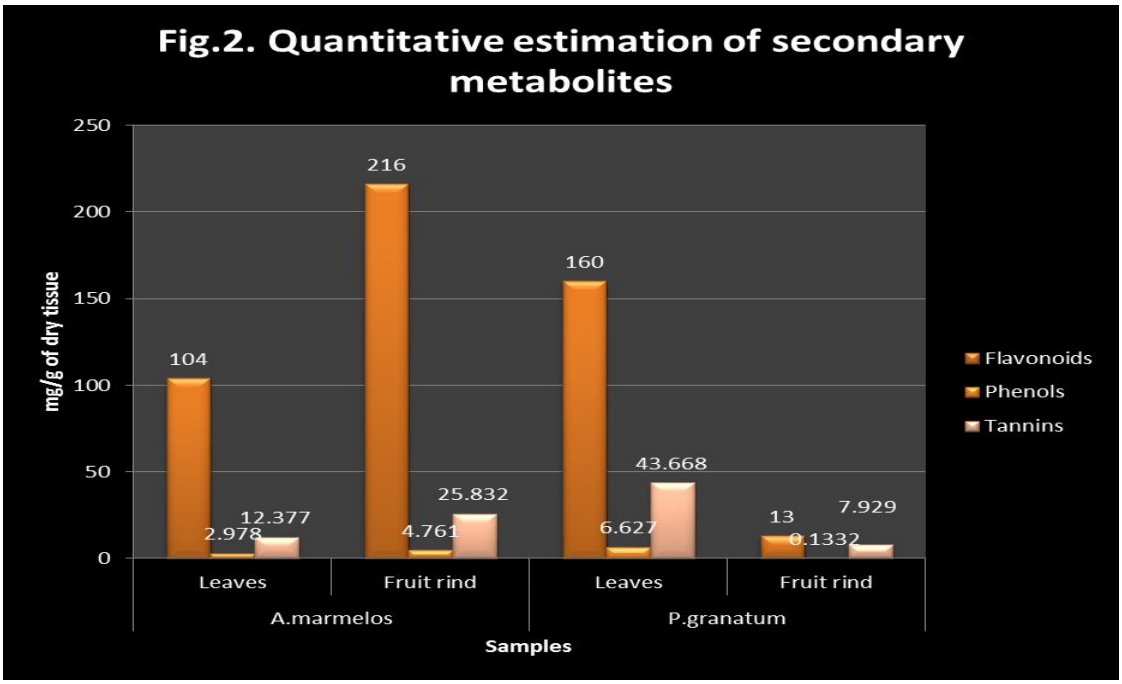
| S.No | Name of the plants | Plant parts tested | Reducing sugars mg/g dry tissue | Sugars mg/g dry tissue | Amino acids mg/g dry tissue | Proteins mg/g dry tissue |
|---|---|---|---|---|---|---|
| 1. | *Aegle marmelos*, (L.) Correa | Leaves | 4.275 | 11.500 | 3.750 | 0.23 |
| | | Fruit rind | 7.625 | 15.750 | 6.250 | 0.38 |
| 2. | *Punica granatum*, L. | Leaves | 7.125 | 19.875 | 3.813 | 0.2 |
| | | Fruit rind | 9.125 | 24.250 | 4.250 | 0.52 |




**QUANTITATIVE ANALYSIS**

**Table 7: Estimation of secondary metabolites**

| S.No | Name of the plants | Plant parts tested | Flavonoids mg/g dry tissue | Phenols mg/g dry tissue | Tannins mg/g dry tissue |
|---|---|---|---|---|---|
| 1. | *Aegle marmelos*, (L.) Correa | Leaves | 104 | 2.978 | 12.377 |
| | | Fruit rind | 216 | 4.761 | 25.832 |
| 2. | *Punica granatum*, L. | Leaves | 160 | 6.627 | 43.668 |
| | | Fruit rind | 13 | 0.1332 | 7.929 |



**REFERENCES**


1. Stary F and Hans S. The National guide to medical herbs and plants. Tiger Books. Int Pub Uk. 1998
2. Del Campo AR, Amiot MJ and Nguyen C. Antimicrobial effect of Rosemary extract. J Food Product. 2000;63:1359 - 1368.
3. Sumathi P. Studies on antimicrobial acivity and phytochemical analysis of some traditional medicinal plants. Ph.D. thesis. 2010.
4. Ghosh SK, Bhaskar Sanyal, Satyabrata Ghosh and Sbhendu Gupta. Screening os some angiospermic plants for antimicrobial activity. Journal of Mycopathological Research. 2000;38(1) :19 – 22
5. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4(7):685 – 688.
6. Panda SK, Thatoi HN and Dutta SK. Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo* L. from similipal biosphere reserve Orissa. Journal of Medicinal Plants Research. 2000;3(4):294-300.
7. Zakaria ZA, Mat Desa A, Ramasamy K, Ahmat N, Mohamed AS, Israf DA and Sulaiman MR. Lack of antimicrobial activities of Dicranopteris linearis extracts and fractions. Afr J Microbiol Res. 2010; 4(1):71 – 75.
8. Sumathi P and Parvathi A. Antimicrobial activity of some traditional medicinal plants. Journal of Medicinal Plants Research. 2010;4(4):316–321.
9. Thiripura Sundari U, Rekha S and Parvathi A. Phytochemical analysis of some therapeutic medicinal flowers. Int J Pharma. 2012;2(3):583.
10. Gnana Sundari S, Rekha S and Parvathi A. Phytochemical evaluvation of three species of *Solanum* L. Int.J. Res. Ayurveda Pharm. 2013;4(3):420–425.
11. Vijay B, Lambole, Krishna Murti, Upendra Kumar, Bhatt Sandipkumar P and Vipul Gajera. Phytopharmacological properties of *Aegle marmelos* as a potential medicinal tree: an overview. International Journal of Pharmaceutical Sciences Review and Research. 2010;5(2): 67-72.
12. Venkatesan D, Karrunakarn CM, Selva kumar S and Palani Swamy PT. Identification of phytochemical constituents of *Aegle marmelos* responsible for antimicrobial activity against selected pathogenic organism. Ethnobotanical Leaf lets. 2009;13:1362-72.
13. Rajan S,Gokila M, Jency P, Brindha P and Sujatha RK. Antioxidant and phytochemical properties of *Aegle marmelos* fruit pulp. International journal of current pharmaceutical research. 2011;3(2): 65-69.
14. Umavathi R and Parvathi A. Sacred trees of temples of Tiruchirappalli, Tamil Nadu –the natural and ecological heritage of India. GJRMI. 2012;1(6):225-233.
15. Mariya Ramya I. A study on secondary metabolites of *Aegle marmelos* (L.) Correa and *Punica granatum* L. M.Sc. thesis. 2012.
16. Irvine FR. WoodyPlants of Ghana – with special reference to their uses. Oxford University press, London. 1961;65.
17. Gamble JS. Flora of the Presidency of Madras. Botanical Survey of India, Calcutta.1957;1 and 2.
18. Matthew KM . The flora of the Tamil Nadu Carnatic. The Diocesan press, Chennai. 1983.
19. Gibbs RD. Comparative chemistry and phylogeny of flowering plants. Tans. Roy Soc canser. 1974; 48.
20. Harborne JB. Phytochemical Methods. A guide to modern techniques of plant analysis. chapman and Hall, London. 1984.
21. Trease GE and Evans WC. Textbook of Pharmacognosy. Sanders, London. 1989. 14th ed. W.B.
22. Lowry OH, Rosedrough NJ, Farr AL and Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193:256 - 275.
23. Moore S and Stein WH. Photometric ninhydrin methods for use in the chromatography of amino acids. J.Biol.Chem. 1948 ;176:367-388.
24. Mahadevan A and Sridhar R. Methods in physiological plant pathology,Sivakami publications, Madras. 1982.
25. Bohm BA and Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawailan vaccinium vaticulatum and V. calycinium. Pacific Sci.1974; 48:458 - 463.
26. Sadasivam S and Manickam A. Biochemical methods for agricultural sciences. Wiley Eastern Ltd., Madras. 1992.

27. Thillai Sivakumar N and Venkataraman R. Phytochemical and pharmacological studies on plant waste materials. Pelagia Research Library. 2010;1(1):1–6.
28. Hameed MA, Faheem Amir, Koay Yen Chin and Shaheedha Fathima. Phytochemical screening, anti- pyrtic and antidiarrhoel activities of the n-hexane and aqueous extracts of the leaves of *Aegle marmelos*. Archives of Pharmacy Practic. 2011;2(3):90-94.