



RESEARCH ARTICLE

**PHYLLANTHUS EMBLICA WOOD AN EFFECTIVE BIO- RESOURCE FOR POTABLE WATER SOFTENING**

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ABSTRACT

An attempt was made to remove the hardness from bore well drinking water collected near sea shore area of Kanyakumari district. About 8 different sample stations were selected in Kanyakumari district. The physico-chemical parameters such as pH, TS, TDS, TSS, acidity, alkalinity, calcium, magnesium, total hardness, chloride, sulphate, phosphate, nitrate, fluoride, of the water samples were analyzed according to the standard APHA methodology. The obtained results were compared with water quality standards prescribed by BIS. All the water samples were not much contaminated and fit for domestic purpose except for an increase in few parameters in one of the selected station near the seashore (siluvai nagar). Treatment with *Phyllanthus emblica* wood reduced the hardness related parameters. 1.5g of wood piece was found to remove 35% of TDS, 82% of calcium, 41% of magnesium and 86% of total hardness from sample S1 in two hours. From the above results it is clear that this wood can be used to treat hard water at house hold level which is contaminated with TDS, Calcium, magnesium and other water related hardness. Ethanolic extract of wood was screened for the presence of potent bioactive phyto compounds using GC-MS analysis and also the antimicrobial activity of the wood was tested against *B. subtilis*, *P. aeruginosa*, *S. aureus* and *E. coli*. Adsorption, chelating nature of wood followed by precipitation process of Amla might have reduced the hardness from drinking water. Hence, this study was conducted to find out the potentiality of *Phyllanthus emblica* wood in softening hard water. Understanding the mechanism of hard water treatment using the above biological material requires further studies.

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INTRODUCTION

Water is a vital element for both the human existence, and all developments (Raju *et al.*, 2011). The property of ground water depends upon geology of a particular area, seasonal changes, composition of dissolved salts depending on the source and from soil surface interaction. The drastic pollution activities over the surface waters largely affect the ground water quality (Mushin *et al.*, 2012). Ground water quality depends on the quality of recharged water, atmospheric precipitation, in- land surface water, and on subsurface geochemical processes. Temporal changes in the origin and constitution of the recharged water, hydrologic and human factors, may cause periodic changes in groundwater quality (Rao, 2006). The key drawback in coastal areas is the depletion of surface water and groundwater; due to saline intrusion and migration of sea water towards land side.

And so, the fresh water aquifers are turned into saline water zones in the coastal deltaic plains (V.E.A. Post, 2004). Monitoring the quality of drinking water at regular time interval is essential to identify the magnitude and pollution load because unsafe drinking water contributes to numerous health problems associated mainly with water borne diseases. According to the physicochemical nature of water, it is broadly classified into soft water and hard water. Soft water is the pure water that can be used for drinking purpose (potable). When higher amount of magnesium and calcium are dissolved in water, it is called as hard water (Basavaraja Simpi *et al.*, 2011). Hard water minerals such as calcium, magnesium, iron and manganese result in scaling problems and serious failures in pipelines of boilers and heat- transfer equipment. However, calcium and magnesium are the most common sources of water hardness (Park *et al.*, 2007). Higher level of calcium in drinking water leads to colorectal, gastric and breast cancer. Taking too much supplemental magnesium can result in symptoms of toxicity, such as fall in blood pressure, abnormal cardiac rhythm, muscle weakness, difficulty breathing and deterioration of kidney function (Yang and Chiu, 1998). Process of purifying drinking water is aimed to improve the potable quality of water both at house hold level and industrial

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level. Commonly adopted water purifying method for domestic purpose includes boiling, filtration, chlorination etc. yet there is always a quest for novel water purifying technique. Traditionally water is purified by adding parts of herbs or stored in copper vessels (Khurana and Sen, 2012). The wood of Amla (*Phyllanthus emblica*) is used to clear small rain ponds in the Indian peninsula to obtain safe and healthy drinking water. This is practically cost less way to afford contaminated water fit for human consumption (Durairasan, 1999).

Hence, this study was focused to assess the quality of bore well water of Kanyakumari district and water hardness removal property of Amla wood. Also to screen its bioactive fraction using spectroscopic GC-MS studies. This may provide an insight in its use in traditional water purification process. Plants containing flavonoids, terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Hostettmann *et al.*, 1977).

## Experimental

### Sampling sites

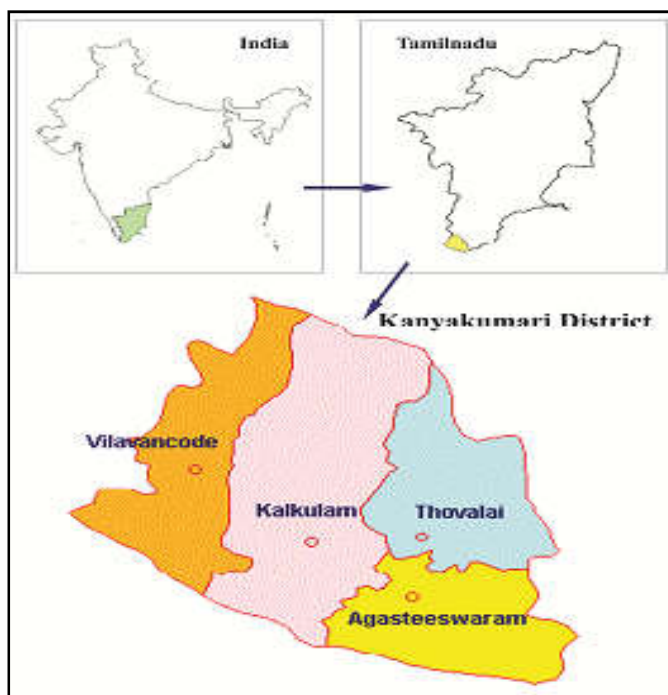


Fig. 1. Study area- Taluks of Kanyakumari district, Tamil Nadu, India

For this study, water samples were collected from four taluks such as Agasteeswaram, Vilavancode, Kalkulam and Thovalai of Kanyakumari district (Fig. 1). This district lies at the southernmost tip of India which is encircled by sea on three sides and with Western Ghats bordering on the northern side. The geographical position lies between 77° 15' and 77° 36' east and 8° 03' and 8° 35' north. In the present study two samples from each taluks, one from the coastal area and the other from inland area were collected. The samples were collected during the month of June. The sampling sites, geographic location and source are given in table 1.

Table 1. Sampling sites

| S.No. | Taluks        | Sampling sites/<br>Geographic locations | Sampling sources | Sampling code |
|-------|---------------|---|------------------|---------------|
| 1     | Agasteeswaram | Siluvai nagar (coastal)                 | Bore well        | S1            |
| 2     |               | James town (inland)                     | Bore well        | S2            |
| 3     | Thovalai      | Boothapandi (inland)                    | Bore well        | S3            |
| 4     |               | Chenbaga Ramanputur (inland)            | Bore well        | S4            |
| 5     | Kalkulam      | Colachel (coastal)                      | Bore well        | S5            |
| 6     |               | Villukuri (inland)                      | Bore well        | S6            |
| 7     | Vilavancode   | Neerodi colony (coastal)                | Bore well        | S7            |
| 8     |               | Melpuram (inland)                       | Bore well        | S8            |

## Methodology

### Physico-chemical characterization of drinking water

Underground bore well water samples from the selected sites were collected in pre cleaned sterilized polyethylene bottles. The water samples were brought to the laboratory and stored at 4°C till use. Temperature and pH of the water were recorded at the time of sample collection using thermometer and pocket digital pH meter respectively. While rest of the parameters such as calcium, magnesium, hardness, alkalinity, chloride, total solids and dissolved solids, were estimated by standard methods prescribed by APHA (2012).

### Collection and Preparation of plant material

Commonly *Phyllanthus emblica* tree is available in almost all the houses of sampling sites. Fresh and Good quality *Phyllanthus emblica* wood were collected from the sampling site-Siluvai Nagar. Wood was cleaned, shade dried, the coat was removed and the wood was crushed using electric mixer and stored in dry air tight container. This was used for further study.

### Effect of treatment with *Phyllanthus emblica* wood

S1 water sample with the higher amount of hardness, TDS, calcium and magnesium sample was treated with raw crushed Amla in static mode. About 100ml of sample of water was taken in 5 conical flask and accurately 0.5g, 1g, 1.5g, 2g, and 2.5g dosage of crushed Amla was added and left for 2 hours. After, the water was filtered through whatmann filter paper no-1. The filtrate was subjected to hardness, calcium and magnesium estimation.

### Phytochemical analysis of *Phyllanthus emblica* wood Ethanol extraction

Cold extract of Amla wood was done. Crushed *Phyllanthus emblica* (100g) were soaked in 200ml of ethanol overnight. The extract was filtered using sterile muslin cloth and filtered through sterile Whatmann no: 1 filter paper. The filtered extract was concentrated using rotary film evaporator. The extract was used for the detection of preliminary phyto-compounds qualitatively and also injected for GC-MS analysis. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology

(NIST). The mass spectrum of the unknown components was compared with the spectrum of the known components that is stored in the NIST library.

#### Antimicrobial activity for ethanolic extract of *Phyllanthus emblica* wood

The solvent extracted from plant material (crude extract) was stored at 4 C until evaluation of antimicrobial activity. Bacterial test organism: A total of 4 bacterial strains, Gram positive: *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative: *Escherichia coli*, *Pseudomonas aeruginosa* were used for the evaluation of antimicrobial activity. The antimicrobial activity of extract was evaluated by determining the zone of inhibition using disc diffusion assay (Rath *et al.*, 1999 and Sahoo *et al.*, 2006). The sterile disc 6mm in diameter were impregnated with 50µl and 100 µl of above extract and placed in inoculated agar. The controls were prepared using the same solvent employed to dissolve the extract. The inoculated plates were incubated at 37 C for 24 hours. The diameter in zone of inhibition was measured at cross angle.

## RESULTS AND DISCUSSION

The findings of the present investigations are summarized as follows.

#### Physico-chemical characterization of drinking water

The quality of drinking water is an essential constituent for all living organisms. The various physico-chemical parameters of bore well water are given in Table 2. From the analyzed results it was found that the quality of the water varied considerably from location to location. The obtained values were compared with BIS (Bureau of Indian Standard) standard values. All the parameters for most of the samples were found within the standard permissible limit except for few parameters for sample S1 (Siluvai nagar) only.

#### pH

In the present study pH value of the samples ranged from 6.51 – 7.58, this lies within the tolerance limit prescribed by BIS (6.5 – 8.5). Alteration in the pH value of natural water occurs due to biological activity and industrial contamination. Similar such results have been reported by Thamaraiselvi *et al.*, (2014).

Table 2. Physico-chemical characterization of bore well water from Kanyakumari district

| S.No | Parameters            | BIS standard    | S1    | S2        | S3    | S4        | S5    | S6        | S7    | S8        |
|------|-----------------------|-----------------|-------|-----------|-------|-----------|-------|-----------|-------|-----------|
| 1    | Color                 | Colorless       | Color | Color     | Color | Color     | Color | Color     | Color | Color     |
|      |                       |                 | Less  | less      | less  | less      | less  | less      | Less  | less      |
| 2    | Odor                  | Unobjectionable | Odor  | Odor      | Odor  | Odor      | Odor  | Odor      | Odor  | Odor      |
|      |                       |                 | Less  | less      | less  | less      | less  | less      | Less  | less      |
| 3    | Taste                 | Agree able      | Salty | Agreeable | salty | Agreeable | Salty | Agreeable | Salty | Agreeable |
| 4    | pH                    | 6.5 – 8.5       | 6.51  | 7.50      | 7.43  | 7.48      | 6.57  | 7.41      | 6.74  | 7.58      |
| 5    | EC (µS/cm)            | -               | 0.41  | 0.11      | 0.48  | 0.13      | 0.23  | 0.16      | 0.17  | 0.29      |
| 6    | TS (mg/L)             | -               | 2100  | 500       | 1000  | 300       | 400   | 100       | 800   | 500       |
| 7    | TDS (mg/L)            | 500– 2000       | 2400  | 300       | 600   | 200       | 600   | 200       | 500   | 400       |
| 8    | TSS (mg/L)            | -               | 300   | 200       | 400   | 100       | 200   | 100       | 300   | 100       |
| 9    | Acidity (mg/L)        | -               | 25    | 25        | 25    | 50        | 25    | 50        | 25    | 50        |
| 10   | Alkalinity (mg/L)     | 200– 600        | 700   | 50        | 500   | 500       | 450   | 200       | 400   | 100       |
| 11   | Calcium (mg/L)        | 75 – 200        | 829   | 161       | 93    | 120       | 136   | 140       | 96    | 100       |
| 12   | Magnesium (mg/L)      | 30 – 100        | 152   | 191       | 27    | 98        | 170   | 146       | 186   | 58        |
| 13   | Total hardness (mg/L) | 300– 600        | 981   | 352       | 120   | 218       | 306   | 286       | 282   | 158       |
| 14   | Chloride (mg/L)       | 250-1000        | 399   | 39        | 214   | 64        | 334   | 29        | 59    | 20        |
| 15   | Sulfate (mg/L)        | 200 - 400       | 281   | 25        | 110   | 50        | 210   | 25        | 40    | 25        |
| 16   | Phosphate (mg/L)      | -               | 1     | 10        | 3     | 7         | 3     | 10        | 3     | 10        |
| 17   | Nitrate (mg/L)        | 45 -100         | 20    | 14        | 14    | 13        | 14    | 14        | 13    | 12        |
| 18   | Fluoride (mg/L)       | 1-1.5           | 1.2   | 1.0       | 1.1   | 1.1       | 1.0   | 1.2       | 1.2   | 1.1       |

Note: All the values are presented as mg/L except for pH and EC.

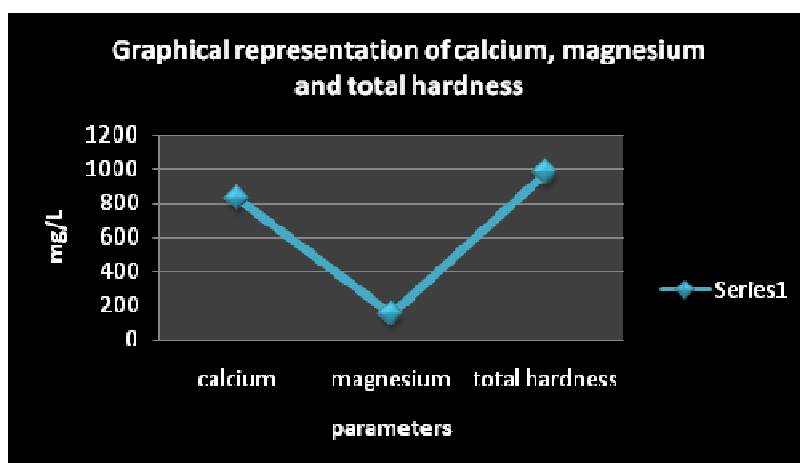


Fig. 2. Graphical representation of calcium, magnesium and total hardness of S1-sample

## Electrical Conductivity

Electrical conductivity value typically depends on the concentration of total dissolved salts in water (Shrinivasa Rao and Venkateswaralu, 2000). The Electrical Conductivity of the water is the sum of ionic conductance of all ionic constituents. Higher EC indicates the enrichment of salts in the ground water. EC of the entire sample was found in the range of 0.29 to 0.41  $\mu\text{S}/\text{cm}$ . Bujar *et al.* (2013) have reported similar such results.

## Solids (TS, TDS, TSS)

Solids in water may be present either in dissolved or suspended form. TDS values are primarily due to carbonates, bicarbonates, chlorides, sulphates, phosphates, nitrates etc (Esmaeili and Johal, 2005). Presence of TDS in water does not cause harm to humans, but higher concentrations leads to heart and kidney diseases. Most of the study has revealed the presence of high dissolved salts in bore well as well as hand pump water compared with open well water. The TS, TDS and TSS values of present investigation are within 100 mg/L to 2100 mg/L; 200 mg/L to 2400 mg/L and 100 mg/L to 400 mg/L respectively. The highest TDS value was observed in S1 and the lowest in S4 and S6. The results were coincided with the findings of Kumaresan *et al.* (2013).

## Acidity and Alkalinity

The acidity values of the samples ranged between 25 mg/L to 50 mg/L. The pH values below 4 causes redness and irritation in eyes. pH below 2.5, damages the epithelium and it is irreversible and extensive (WHO, 1996). The potential of water to neutralize a strong acid is called alkalinity; hence alkalinity of water is due to existence of bicarbonates, carbonate and hydroxide compounds of calcium, sodium and potassium (Patil and Patil, 2009). The alkalinity values of the samples were observed between 50 mg/L to 700 mg/L. The highest amount of alkalinity was found in sample S1. A similar result has been reported by Sartaj Ali *et al.* (2013).

## Calcium, Magnesium, Total hardness

Element calcium enters into the water by the leaching process of rocks and its high content may cause hardness of water (Karikari and Ondi, 2004). Examined values for calcium hardness of water samples ranged between 93 mg/L to 829 mg/L. Sample S1 was found to contain the highest amount of calcium than the tolerance limit. Magnesium is a limiting factor for the growth of phytoplankton and chlorophyll formation (Dagaonkar, Saksena, 1992). The magnesium values of the present investigation was between 27 mg/L to 152 mg/L. S1, S2, S5, S6 and S7 samples had magnesium greater than the tolerance limit. The hardness of water caused by a variety of dissolved polyvalent metallic ions, predominantly Ca & Mg ions. The hardness of the sample is between 120 mg/L to 981 mg/L. The analytical results indicate that the water in the study area is soft to very hard. S1 sample has the highest water hardness property. The S1 sample location is near sea shore, sea water intrusion may be the reason for such high hardness.

Similar such results have been reported by Kannan and Mani, (2014).

## Chloride

In all the water samples the amount of chloride was within the standard limit. The values of the chloride ranges from 20 mg/L to 399 mg/L. Similar results have been comparable to Kumaresan *et al.* (2013).

## Sulphate

Sulphate is one of the major anion occurring in natural waters. The higher amount of sulphate -could cause a cathartic action on human beings and also cause respiratory Problems (Sujitha, *et al.*, 2012). All the samples for sulphate fall within the limit.

## Phosphate

Use of pesticides and fertilizers for crop cultivation increases the phosphate concentration in water resources. Consumption of water contaminated with higher amount of phosphate leads to phosphine gas production in gastro-intestinal tract on reaction with gastric juice. This could lead to the death of the consumer. In the present study phosphate ranged from 1mg/L to 10 mg/L. Similar view has been reported by Shah *et al.* (2012).

## Nitrate

Nitrate is one of the important plant nutrients in soil. While nitrate occurs naturally in some ground water, in most cases higher levels are thought to occur due to human activities. Higher level of nitrate in potable water causes methemoglobinemia in infants. The amount of nitrate in the analyzed samples ranged with in between 12 mg/L to 20 mg/L. Kumaresan *et al.* (2013) have reported similar results in their study.

## Fluoride

Fluoride is broadly scattered element and a member of the halogen family. It is naturally found in the rocks, coal, clay and soil (Public Health statement of Fluorides department of Health and Human Service, 2003; Fazal Hoque, 2002). The natural sources for fluoride in drinking water are fluorite, apatite, rock phosphate and topaz. The amount of fluoride present in the entire samples was within the standard limit. Amaliya and Sugirtha, (2013) have stated the similar views.

## Effect of *Phyllanthus emblica* wood on the removal of Ca, Mg and Hardness

Removal of calcium, magnesium and total hardness are depicted in Table 3. The removal of Ca, Mg and hardness were increased when increase the dosage of Amla up to 1.5g. After that no considerable reduction of Ca, Mg and hardness were noticed. It suggests that 1.5g of plant material is an optimum dosage. This optimum dosage removed about 82% of calcium, 41% of magnesium and 86% of total hardness from the water sample S1.

**Table 3. Effect of *Phyllanthus emblica* wood on removal of Ca,Mg and Hardness (S1)**

| S.No | Dosage (g) | % of reduction - (S1) |           |                |
|------|------------|-----------------------|-----------|----------------|
|      |            | Calcium               | Magnesium | Total hardness |
| 1    | 0.5        | 76                    | 40        | 71             |
| 2    | 1          | 79                    | 40        | 73             |
| 3    | 1.5        | 82                    | 41        | 86             |
| 4    | 2          | 62                    | 41        | 74             |
| 5    | 2.5        | 62                    | 39        | 74             |

**Table 4. Preliminary phytochemical analysis of ethanol extracted *Phyllanthus emblica* wood**

| S.No | Phytochemicals | Presence/ Absence |
|------|----------------|-------------------|
| 1    | Alkaloids      | +                 |
| 2    | Flavonoids     | -                 |
| 3    | Steroids       | +                 |
| 4    | Tannin         | +                 |
| 5    | Quinone        | -                 |
| 6    | Protein        | -                 |
| 7    | Terpenoids     | +                 |
| 8    | Saponins       | -                 |
| 9    | Glycoside      | +                 |

**Table 5. GC-MS analysis of ethanol extracted *Phyllanthus emblica* wood**

| S.No | Compound name             | Activity                                  | Molecular weight | Molecular formula |
|------|---------------------------|---|------------------|-------------------|
| 1    | Tridecyclic acid          | Antimicrobial/ Anticancer                 | 214              | C12H26O2          |
| 2    | Pentadecyclic acid        | Antifungal/Antibacterial                  | 242              | C15H30O2          |
| 3    | Myristic acid             | Pharmacological                           | 228              | C14H28O2          |
| 4    | 7,10-Hexadecadienoic acid | Antioxidant                               | 266              | C17H30O7          |
| 5    | Oleic acid                | Anticancer                                | 282              | C18H34O2          |
| 6    | 9,10- Epoxyctadecon       | Antimicrobial                             | 284              | C18H36O2          |
| 7    | Oxirane                   | Pharmacological                           | 298              | C19H38O2          |
| 8    | Butoxyacetic acid         | haemolytic                                | 132              | C6H12O3           |
| 9    | Squalene                  | biological and pharmacological activities | 410              | C30H50            |

**Table 6. Antibacterial activity ethanolic extract of *Phyllanthus emblica* wood**

| S.No. | Microorganism                 | Strain +/- | Concentration ( $\mu$ l) | Zone of inhibition (mm) |
|-------|-------------------------------|------------|--------------------------|-------------------------|
| 1     | <i>Escherichia coli</i>       | -ve        | 50                       | 5                       |
|       |                               |            | 100                      | 10                      |
| 2     | <i>Pseudomonas aeruginosa</i> | -ve        | 50                       | 6                       |
|       |                               |            | 100                      | 11                      |
| 3     | <i>Staphylococcus aureus</i>  | +ve        | 50                       | 12                      |
|       |                               |            | 100                      | 22                      |
| 4     | <i>Bacillus subtilis</i>      | +ve        | 50                       | 6                       |
|       |                               |            | 100                      | 12                      |

After the treatment white precipitate of salt has seen in the vessel during filtration. It shows the ability of Amla would precipitate the hardness causing chemical species thereby the Ca, Mg and hardness has been reduced. Chelating process followed by precipitation process of Amla might have reduced the hardness from drinking water. Also chelating property of *Phyllanthus emblica* wood has been reported and recorded by Satish *et al.* (2012).

#### Phytochemical analysis of ethanol extracted *Phyllanthus emblica* wood and GC-MS characterization

The preliminary phytochemical analysis showed the presence of alkaloids, steroids, tannin, terpenoids and glycosides and is presented in Table 4 and the activity of phytochemical compounds are depicted in Table 5. The GC-MS analysis revealed the presence of 9 compounds and presented in Table-5. From the phytochemical analysis of the Amla wood it is

clear that it has an important antioxidant compound such as hexadecadienoic acid. This antioxidant must have reduced the hardness species like  $\text{CO}_3^-$ ,  $\text{HCO}_3^-$ ,  $\text{PO}_3^-$ ,  $\text{SO}_4^-$  etc. Similar results have been reported Trease *et al.*, 1978. Thereby, it must have removed the chemical species, which results in the highest removal of Hardness.

#### Antimicrobial activity for ethanolic extract of *Phyllanthus emblica* wood

The zone of inhibition is shown in Table 6. The observed activity may be due to the presence of potent phytoconstituents in the extracts (Ikram & Innannual 1980a, 1980b) and present investigation on the phytochemical analysis refers to the presence of alkaloids, glycosides, saponins, tannins, triterpenoids, fixed oils and simple phenol compounds which might be responsible for its antimicrobial properties. Among the extracts assayed the ethanolic extract *P.emblica*

exhibited good activity against *S. aureus* at 100 µl for example, 22 mm was recorded as diameter zone of inhibition. This was followed by 12 mm of *B. subtilis*, *P. aeruginosa* of 11 mm and 10 mm of *E. coli* observed. This antimicrobial activity was achieved by the presence of Tridecyclic acid and Pentadecyclic acid. Naturally occurring substance of plant origin have been reported to inhibit the growth of microorganisms. Plants extracts have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to microbial activities (Irobi 1992).

## Conclusion

From the investigation Amla wood pieces can be used for the removal of hardness from the drinking water. The colour and odour of the hard water sample (S1- siluvai nagar) remained the same before and after treatment with *Phyllanthus emblica* wood pieces. Before treatment the water was slightly salty and after treatment no taste was observed. The deterioration in ground water quality is due to intrusion of sea water and the presence of salt in it made the water hard. Hard water is unfit for drinking and is the major cause for many health problem particularly kidney stone. Hardness in water due to salt is very difficult to remove. Prolonged treatment such as increase in dosage after the optimum dose and increase in contact time released tannin compounds from the bark which resulted in colour change in the water samples from colorless to light brown. Hence, two hour contact time is enough to remove hardness. Scientific studies have revealed that use of *Phyllanthus emblica* wood pieces to treat water has altered the nature of drinking water and it is good for health. Further studies are required to reveal the mechanism by which it alters the characteristics of drinking water for potability.

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