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ANTIPATHOGENIC EFFICACY OF METHANOLIC LEAF EXTRACT OF *CINNAMOMUM TAMALA* (BUCH.-HAM.) AND *AEGLE MARMELOS* (L.) WITH THEIR NUTRITIONAL POTENTIALITY

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ABSTRACT
Antipathogenic efficacy of methanolic leaf extract of *Cinnamomum tamala* (Buch.-Ham.) and *Aegle marmelos* (L.) through inhibiting the growth of *Staphylococcus aureus* (MTCC 3160), *Salmonella typhi* (MTCC 3216) and *Proteus mirabilis* (MTCC 7837) the causative pathogens of food poisoning, boils, abscesses, wound infection, pneumonia, toxic shock syndrome, typhoid fever, urethitis, cystitis, pylonephritis and prostatitis has been investigated. All the strains were affected by methanolic leaf extract of *C. tamala* and *A. marmelos* in agar diffusion method and broth dilution method. The MIC values in agar diffusion method were 2.5 mg/mL against *S. aureus* for extract of both plants and 5 mg/mL, 1.25 mg/mL, against *P. mirabilis* for the extract of *C. tamala* and *A. marmelos* respectively. The MIC values in broth dilution method were 2.5 mg/mL against *S.aureus* for both plants extract, 4 mg/mL, 1 mg/mL against *P.mirabilis* and 9 mg/mL, 10 mg/mL against *S. typhi* for *C. tamala* and *A. marmelos* respectively. The nutritional value, phytochemical contents and inorganic substance content of *C. tamala* is higher than *A. marmelos*.

INTRODUCTION

Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multicellular parasites. These diseases are also called as communicable or transmissible diseases since they can be transmitted from one person to another via a vector results in the symptoms of disease (Solanki, 2010).

Three common pathogenic bacteria have been tested. *P. mirabilis* is known to cause urethitis, cystitis, pylonephritis, prostatitis and pneumonia (Todar, 2012). *Staphylococcus* species are predominant among the organisms that are responsible for infective complications following surgical vascular grafts or the implantation of prosthetic devices (De-Lalla, 1999). *Staphylococcus aureus* is a facultative anaerobic, gram positive bacterium, which causes food poisoning and usually grows on the nasal membrane and skin. It is also found in the gastrointestinal and urinary tracts of warm-blooded animals. It also causes boils, abscesses, wound infection, pneumonia, toxic shock syndrome and other diseases (Cheesbrough, 2000). Typhoid fever is predominantly caused by *S. typhi* (Crump *et al*., 2004) and is a global infection (Nagshetty *et al*., 2010).

Plants are rich in secondary metabolites such as tannins, alkaloids, flavonoids, phenols, etc, which are responsible for therapeutic activities (Rabe and Vnstoden, 2000). The review of literature revealed that considerable contributions have been made on medicinal plants by many workers (Dadsena *et al*., 2013; Kullu *et al.,* 2013*;* Kumar *et al.,* 2013*;* Kumar *et al*., 2013a; Mahato *et al.,* 2013*;* Tabassum *et al.,* 2013*;* Toppo *et al.,* 2013*;* Sahu *et al.,* 2013).

Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action (Barbour *et al*., 2004; Ahmad and Aqil, 2007). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999). Medicines obtained from plants are relatively safer than synthetic alternative (Iwu *et al*., 1999; Idu *et al*., 2007). Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds (Tomoko *et al*., 2002).

Aegle marmelos commonly known as bael, belonging to the family rutaceae and *Cinnamomum tamala* belonging to family lauraceae have been tested against the pathogenic bacteria.These plants are frequently used as folk medicine for various treatments (Chopra *et al*., 1956; Kirtikar and Basu, 1995; Rao, 2008). The present study is an attempt to evaluate the potentiality of methanolic leaf extract on *Proteus mirabilis, Salmonella typhi* and *Staphylococcus aureus*.

MATERIALS AND METHOD

Collection of plant material

The fresh tender leaves of *Aegle marmelos* and *Cinnamomum tamala* were collected from Ranchi (23º21' 0" N LR, 85º20'

0" E L), washed and disinfected with 0.1% $HgCl_2$ solution and shade dried. Dried material was then powdered in an electric grinder and sieved (Jonani and Sondhi, 2002).

Extract preparation

50g of the powder was subjected to extraction by soxhlet using methanol. The extract obtained was filtered, concentrated in a rotary flash evaporator at 45ºC, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

Phytochemical analyses

Ash content analysis was done following WHO (1998). The amount of crude fiber was determined following Watanables and Olsen (1965). The moisture content was determined in terms of the loss in weight of the plant material on overnight heating at 150ºC (Sadasivam and Manickam, 1996). Total phenol was determined by Folin-Ciocalteau reagent, following Ramamoorthy and Bono (2007). The tannins content was quantified as percentage following the procedure and formula given in the quality control methods for medicinal plant materials (WHO, 1998). Aluminium chloride colorimetric method was used with some modifications to determine flavonoids content (Lin and Tang, 2007). Alkaloid was determined by the method used by Helrich (1990). Saponin content was determined following Obdoni and Ochuko (2001).

Nutritive value

Crude fat, carbohydrate and protein were quantified following previously published standard tests (Watanble and Olsen 1965; Jayarama, 2005), and nutritive values were calculated following Nile and Khobragade (2009).

Anti-bacterial analysis

Test Microorganisms

Proteus mirabilis MTCC 7837, *Salmonella typhi* MTCC 3216 and *Staphylococcus aureus* MTCC 3160 used during the present experiment were procured from Hi-media Laboratories (Mumbai, India).

Agar diffusion method

Following Threlfall *et al*. (1999) the agar plates were prepared and wells were made in the plate. Each plate was inoculated with 18 hours old cultures of the selected bacteria and spread evenly on the plate. After 20 minutes, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37ºC for 24h and the diameter of inhibition zones were noted.

Broth dilution method

As proposed by Walker (2000) the tubes containing the culture media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 hours old cultures (100ìL, 10^4cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24h; the growth and hence the MIC was measured at 660nm.

RESULTS AND DISCUSSION

Phytochemical profile

The results on phytochemical analyses of *C. tamala* and *A. marmelos* leaf have been represented in Fig. 1, 2 and 3. The result reveal that moisture content is higher in *A. marmelos* leaf than *C. tamala* and ash content is lower in *A. marmelos* leaves than *C. tamala* leaves.

Physicochemical analysis from leaves of various medicinal plants has been reported by various workers from time to time. Indrayan *et al*. (2005) reported 8.20 g/100g ash, 57.90 g/100g moisture and 7.20g/100g crude fiber in *A. hetrophyllus* leaves. Nasiruddin *et al*. (2012) detected 2.84 ± 0.04%, 1.33 ± 0.02%, 3.50 ± 0.03% total ash, 91.60 ± 0.20%, 82.90 ± 0.74%, 85.05 ± 0.50% moisture and 0.94 ± 0.06%, 3.11 ± 0.05%, 2.41 ± 0.05% crude fiber in *Rumex crispus*, *Medicago denticulate* and *Taraxicum officinale* respectively.

The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. Ash usually represents the inorganic part of the plant (Vermani *et al*., 2010). Nutritionally, fiber is beneficial to human body, since it has been reported that food fiber aids absorption of trace elements in the gut (Kelsay, 1981) and reduce absorption of cholesterol (Le - Veille and Sanberlich, 1966). Fiber aids bowel movement of gut (Abolaji *et al*., 2007). Aravind *et al*. (2013) reported, fiber of *Carica papaya* is able to bind cancer-causing toxins in the colon and keep them away from the healthy colon cells. The fibers provide synergistic protection for colon cells from free radical damage to their DNA. *C. tamala* and *A. marmelos* leaves contain higher amount of ash and crude fiber (Fig. 1) compared to the above plants thus *C. tamala* and *A. marmelos* leaf are likely to contain higher amount of inorganic constituent and dietary fibers.

The results on phytochemical analysis of the leaf samples of *C. tamala* and *A. marmelos* is presented in Fig - 3. The result revealed that polyphenols is highest (16.7 ± 0.7 g/100g), flavonoid is lowest (1.0 ± 1.01 g/100g) in *C. tamala* and polyphenols is highest (6.7 ± 0.61 g/100g), alkaloid is lowest (2.3 ± 0.42 g/100g) among all the studied phytochemicals. Aliyu *et al*. (2008) reported 0.110 ± 0.002 g/100g, 0.966 ± 0.030 g/100g, 1.440 ± 0.002 g/100g, 7.270 ± 0.009 g/100g and 2.600 ± 0.200 g/100g alkaloids, 8.000 ± 0.280g/100g, 8.766 ± 0.020g/100g, 16.30 ± 0.042g/100g, 18.23 ± 0.040 g/100g and 9.466 ± 0.060 g/100g flavonoids, 0.533 ± 0.020 g/100g, 2.500 ± 0.014 g/100g,0.900 ± 0.020 g/100g, 2.320 ± 0.001 g/100g and 1.066 ± 0.020 g/100g saponins, 0.566

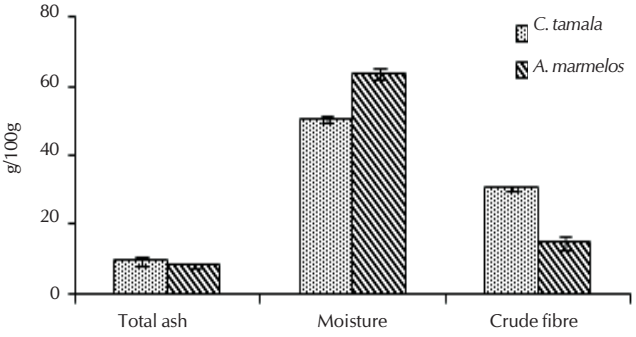

Figure 1: Physicochemical composition of *C. tamala* and *A. marmelos* leaf in g/100g (M ± SD; n = 3).

± 0.010 g/100g,1.250 ± 0.009 g/100g, 0.520 ± 0.200 g/100g, 1.030 ± 0.014 g/100g and 1.140 ± 0.001 g/100g phenols in *Anchomanes difformis, Anisopus mannii, Pavetta crassipes, Stachytarpheta angustifolia* and *Vernonia blumeoides* respectively. Manikandan *et al.* (2010) reported 10.0 mg/g and 13.0 mg/g tannin in *Ruelli atuberosa* L. and *Dipteracanthus patulus* (Jacq.) respectively. Soladoye and Chukwuma (2012) reported tannin (4.98%) in *Cissus populnea*. Khan *et al.* (2011) reported tannin content 15.75% in *M. rubicaulis,* 14.16%, *W. fruticosa,* 13.4% in *C. grata*, 12.33% in *V. cotinifolium*, 11.2% in *E. hirta*, 10. 56 % in *B. Papyrifera* and 10.2% in *P. harmala*.

The total phenolic content of *Cinnamomum tamala* and *Aegle marmelos* 16.7 ± 0.7 g/100g and 6.7 ± 0.42 g/100g respectively have been found highest among most of the plants studied. Tannins, alkaloids, saponins, flavonoids, and sterols have been found active against several pathogenic bacteria (Kennedy and Wightman, 2011, Choudhury *et al*., 2013). Tannins form irreversible complexes with prolene rich protein resulting in the inhibition of cell wall synthesis (Mamtha *et al*., 2004).

Flavonoids inhibit several enzymes, chelate certain metal cations, affect protein phosphorylation (Middleton and Kandaswami, 1994) and have variety of effects on membrane - linked processes (Smith, 1996) including the enhancement of metal- induced lipid peroxidation (Sakihama *et al*., 2002). Alkaloids possess anti-oxidizing effects, thus reduces the nitrate generation which is useful for protein synthesis, suppresses the transfer of sucrose from stomach to small intestine. Isaac and Chinwe (2001) reported that alkaloids are responsible for the antibacterial activity.

Nutrition potentiality

The result of nutritional potentiality of *C. tamala* and *A. marmelos* leaves have been represented in fig – 2 and table - 1. The results reveal that carbohydrate content is higher in *A. marmelos* leaves (10.5 ± 0.3g/100g) than *C. tamala* leaf (9.5 ± 0.5g/100g) and fat content is lower in *A. marmelos* leaves (1.7 ± 0.5g/100g) than *C. tamala* leaves (6.0 ± 0.5g/100g).

Indrayan *et al.* (2005) reported 19.70% carbohydrate, 5.70% protein and 2.50% crude fat in *A. hetrophyllus* leaves. Bukhsh et al. (2007) reported 18.9 ± 4.2%, 16.9 ±1.1%, 15.9 ± 1.3% carbohydrate and 21.87 ±4.7% crude protein in *Carthamaus oxyacantha, Eruca sativa* and *Plantago ovate* leaves respectively. The fat conent was 6.6 ± 1.3% in *Eruca sativa* leaves but fat was not found in *Carthamus oxyacantha* and *Plantago ovate* leaves (Bukhsh *et al.,* 2007). Nasiruddin *et al.* (2012) reported 1.82 ± 0.03%, 5.99 ± 0.02%, 2.74 ± 0.01% crude protein and 0.30 ± 0.01%, 0.14 ± 0.03%, 0.21 ± 0.02% crude fat in *Rumex crispus, Medicago denticulate* and *Taraxicum officinale* respectively.

Since carbohydrate constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of animal life (Ebun-Oluwa and Alade, 2007). Proteins contain amino acids utilized by the cells of the body to synthesize all the numerous proteins required for the function of the cell and also to furnish energy (Robinson, 1978). Due to low level of crude fat in the leaves of *A. marmelos* and *C. tamala*, the leaves can be consumed in diet of those people suffering from overweight or obesity (Nasiruddin *et al.,* 2012).

The calculated nutritional value is higher (143.5 ± 0.53 Kcal/100g) in *C. tamala* than *A. marmelos* (82.5 ± 0.74 Kcal/ 100g). Nasiruddin *et al.* (2012) reported total energy 21.15 Kcal, 55.05 Kcal and 48.46 Kcal in *Rumex crispus, Medicago denticulate* and *Taraxicum officinale* respectively. Indrayan *et al.* (2005) reported 124.10 cal/100 g nutritive values of *A. hetrophyllus* leaves. The nutritional values of indigenous fruits and vegetables such as *Cucumis sativus*, *Pangium edule, Brasssica oleraceae, Spinacia oleraceae, Sinapis alba* have been reported as 15 kcal, 227 kcal, 22 kcal, 29 kcal, 34 kcal respectively (Hoe and Siong, 1999).

Table 1: Nutritional value of *C. tamala* and *A. marmelos* (M ± SD; n = 3).

Nutritional value	*C. tamala*	*A. marmelos*
	143.5 ± 0.53 Kcal/100g	82.5 ± 0.74 Kcal/ 100g

Since *C. tamala* and *A. marmelos* leaves contain high amount of carbohydrate, protein, fat (Fig. 2) and nutritional value comparing with the above plants, thus leaves of *C. tamala* and *A. marmelos* can be used as fodder.

Antibacterial analysis

The pathogenic efficacy of methanolic extract of *C. tamala* and *A. marmelos* leaves were quantitatively assessed on the basis of zone of inhibition (ZOI) in mm (Table 2) following the agar disk diffusion method and minimum inhibitory concentration by broth dilution method. The test organisms were inoculated with standard antibiotic: gentamycin to compare the efficacy of leaf extract for their microbial properties (Table 3). In the present investigation the extracts were found

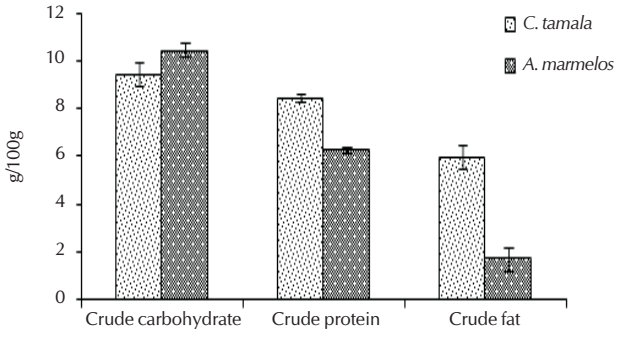

Figure 2: Composition of fat, protein and carbohydrate and nutritive value from *C. tamala* and *A. marmelos* leaf in g/100g (M ± SD; n = 3).

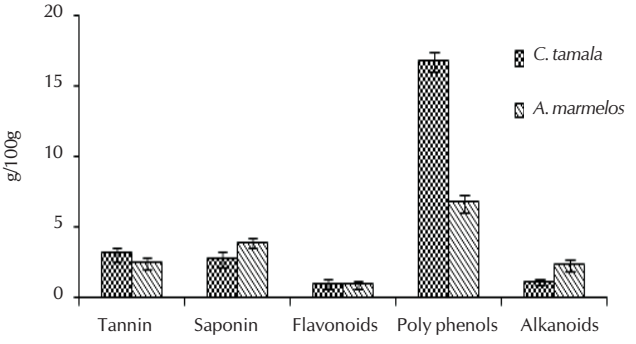

Figure 3: Phytochemicals from *C. tamala* and *A. Marmelos* leaf in g/100g (M ± SD; n = 3).

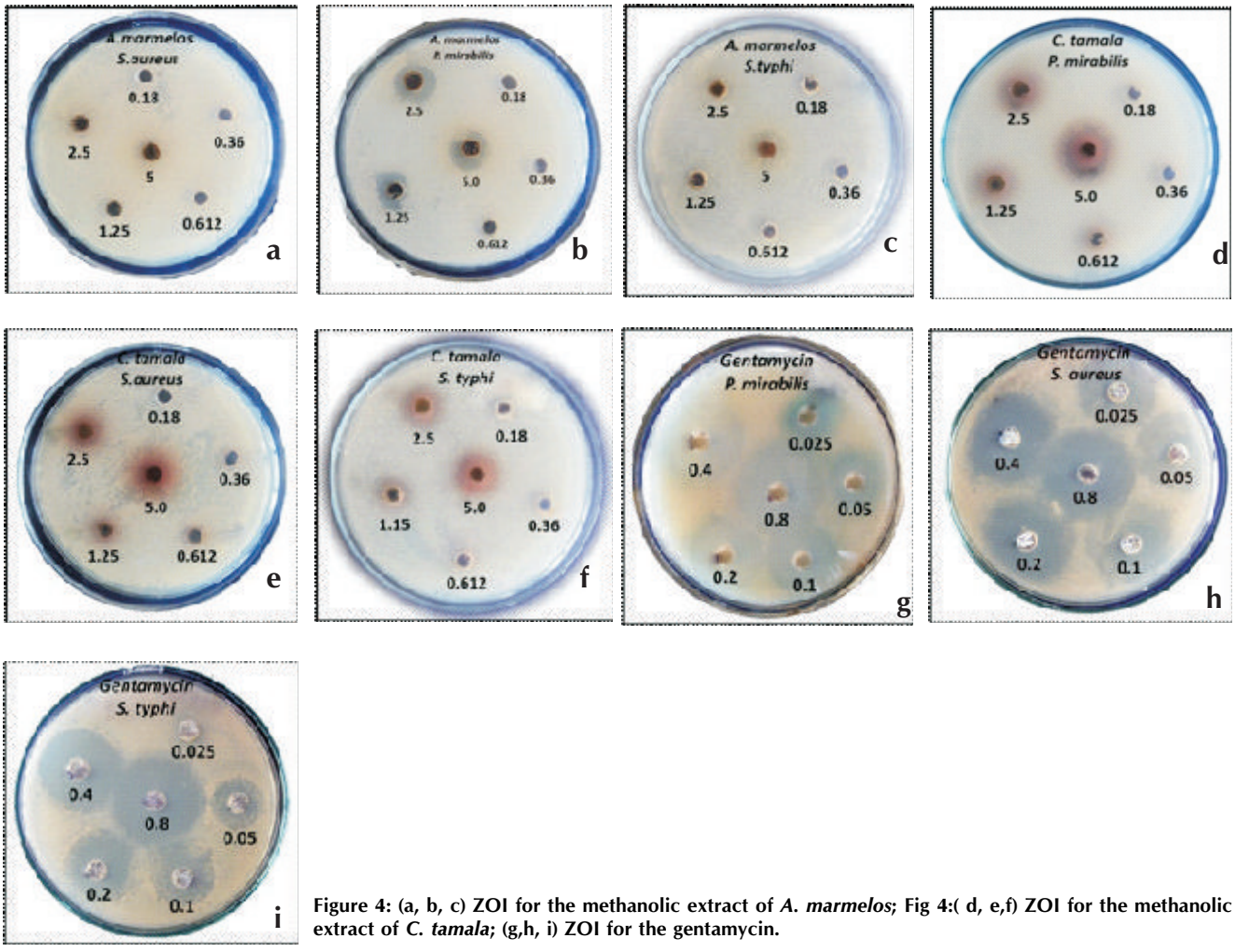

Figure 4: (a, b, c) ZOI for the methanolic extract of *A. marmelos*; Fig 4:(d, e,f) ZOI for the methanolic extract of *C. tamala*; (g,h, i) ZOI for the gentamycin.

to be effective against all the pathogens. The ZOI observed for the methanolic extract and gentamycin using agar diffusion method is represented in Fig. 4a, 4b, 4c, 4d, 4e, 4f and Fig. 4g, 4h, 4i respectively. The broth dilution method showed more pronounced antimicrobial activity through 100% inhibition of all the pathogens in the range of 1.25-10mg/mL concentration (Fig. 5a, 5b, 5c, 5d, 5e and 5f). The minimum inhibitory concentration (MIC) obtained by broth dilution method for *P. mirabilis, S. aureus* and *S. typhi* were in the range of 1mg/mL-10 mg/mL. Kothari *et al*. (2011) worked out on different extract of *A. marmelos* and found 10 ± 0.3 mm-22 ± 0.6 mm zone of inhibition against *S. aureus, S. typhi, P. mirabilis* and other pathogenic bacteria species respectively and also said that methanolic extract was more effective than other extracts. Essawi and Srours (2000) reported that methanolic leaf extract was more effective compared to chloroform and aqueous extract because of chemical constituents which are either polar or non polar and can be effectively extracted only through the organic solvent medium. *Cinnamomum tamala* possess antibacterial activity due to the presence of certain phenolic compound such as cinnamic aldehyde and such as eugenol and cinnamic acid (Baratta *et al*., 1998). An important characteristic of leaf extract and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structure and rendering them

Table 2: The zone of inhibition and MIC (in mm) of methanolic leaf extract of *C. tamala* and *A. marmelos*.

Concentration	*C. tamala*			*A. marmelos*		
(mg/mL)	*P. mirabilis*	*S. aureus*	*S. typhi*	*P. mirabilis*	*S. aureus*	*S. typhi*
0.18	-	-	-	-	-	-
0.36	-	-	-	-	-	-
0.612	-	-	-	-	-	-
1.25	-	-	-	6	-	-
2.5	-	6	-	9	4	-
5	10	6	-	10	5	-
MIC (mg/mL)	5	2.5	-	1.25	2.5	-

Table 3: The zone of inhibition and MIC (in mm) of Gentamycin against the test organism.

Concentration (mg/mL)	*S.typhi*	*S.aureus*	*P.mirabilis*
0.025	2	13	9
0.05	13	18	13
0.10	16	21	18
0.20	21	25	21
0.40	25	27	25
0.80	27	34	27
MIC(mg/mL)	00.25	00.25	00.25

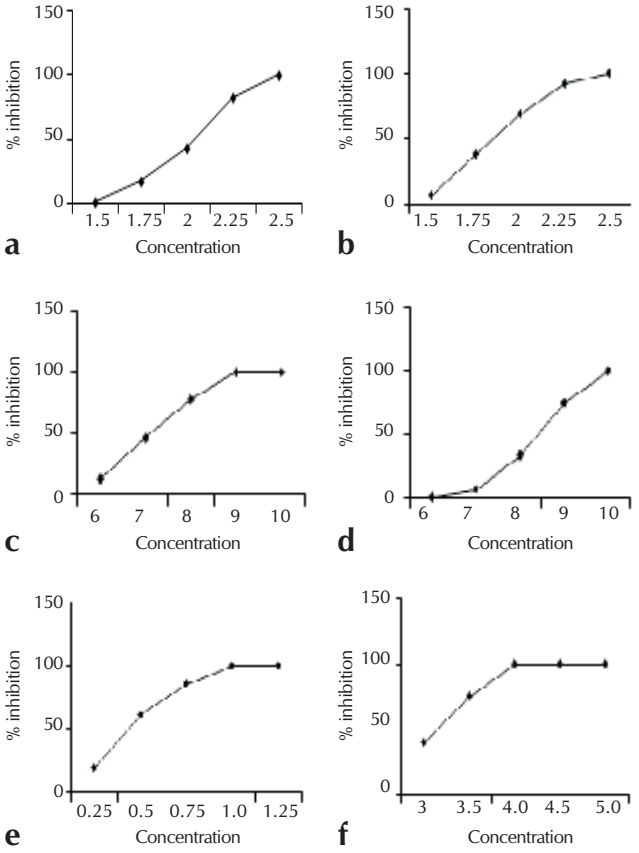

Figure 5: (a) and (b) Inhibition % of *S.aureus,* (c) and (d) Inhition % of *S.typhi,* (e) and (f) Inhibition % of *P. mirabilis* in broth dilution method for Methanolic leaf extract of *C. tamala* and *A. marmelos* respectively.

more permeable (Sikkema *et al*., 1994). Extensive leakages from bacterial cells or exits of critical molecules and ions will lead to death (Denyer and Hugo, 1991). The antibacterial activity *A. marmelos* leaf extract is due to presence of active phenolic compound eugenol and cuminaldehyde because these compounds have already shown their activity against various bacterial strains (Duke, 1992) the mechanism of action may be the blockage of protein synthesis either at transcription or at trainslation level and inhibition of peptido-glycan synthesis at membrane level (Rajan and Jeevagangai, 2009).

The present study suggests antibacterial property of *Cinnamomum tamala* and *A. Marmeos* leaf extract, which inhibits the growth of pathogenic bacteria *S. aureus, S.typhi and P.mirabilis* causative agent food poisoning boils, abscesses, wound infection, pneumonia, toxic shock syndrome, typhoid fever and urethitis, cystitis, pylonephritis, prostatitis and pneumonia disease, and can be used as new drug for therapy.

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