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

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KEYWORDS
Cinnamomum tamala
Aegle marmelos
Antipathogenic
Phytochemical
Nutritional

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 urethritis, cystitis, pyleonephritis, 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 Vnstonden, 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% HgCl₂ 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 45°C, 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 150°C (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 (Watanabe and Olsen 1965; Jayarama, 2005), and nutritive values were calculated following Nile and Khorbagade (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 37°C 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 (100µL, 10⁴cfu). 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 37°C on a shaker with 140 rpm for 24h; the growth and hence the MIC was measured at 660nm.

**RESULTS AND DISCUSSION**

**Figures**

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 diffomis, Anisopus mnnii, Pavetta crassipes, Stachytrapheta angustifolia and Vernonnia blumeoides respectively. Manikandan et al. (2010) reported 10.0 mg/g and 13.0 mg/g tannin in Ruellia aberosa 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 Cinnamonum 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 Kendawaswi, 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. heterophyllus 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 Carthamus oxyacantha, Eruca sativa and Plantago ovate leaves respectively. The fat content 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 (Ebu-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. heterophyllus leaves. The nutritional values of indigenous fruits and vegetables such as Cucumis sativus, Pangium edule, Brassica 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).

<table>
<thead>
<tr>
<th>Nutritional value</th>
<th>C. tamala</th>
<th>A. marmelos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcal/100g</td>
<td>143.5 ± 0.53</td>
<td>82.5 ± 0.74</td>
</tr>
<tr>
<td>Kcal/100g</td>
<td>21.15 Kcal</td>
<td>55.05 Kcal</td>
</tr>
<tr>
<td>Kcal/100g</td>
<td>48.46 Kcal</td>
<td>48.46 Kcal</td>
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</table>

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...
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 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 \textit{P. mirabilis}, \textit{S. aureus} and \textit{S. typhi} were in the range of 1mg/mL-10 mg/mL. Kothari et al. (2011) worked out on different extract of \textit{A. marmelos} and found 10 ± 0.3 mm-22 ± 0.6 mm zone of inhibition against \textit{S. aureus}, \textit{S. typhi}, \textit{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. \textit{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

![](image)

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

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>\textit{C. tamala}</th>
<th>\textit{P. mirabilis}</th>
<th>\textit{S. aureus}</th>
<th>\textit{S. typhi}</th>
<th>\textit{A. marmelos}</th>
<th>\textit{P. mirabilis}</th>
<th>\textit{S. aureus}</th>
<th>\textit{S. typhi}</th>
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<tbody>
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<td>9</td>
<td>4</td>
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<td>6</td>
<td>10</td>
<td>5</td>
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</tr>
<tr>
<td>MIC (mg/mL)</td>
<td>5.0</td>
<td>2.5</td>
<td>1.25</td>
<td>2.5</td>
<td>-</td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>\textit{S. typhi}</th>
<th>\textit{S. aureus}</th>
<th>\textit{P. mirabilis}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>2</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>0.05</td>
<td>13</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>0.10</td>
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<td>21</td>
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<tr>
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<td>21</td>
<td>25</td>
<td>21</td>
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<tr>
<td>0.40</td>
<td>25</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>0.80</td>
<td>27</td>
<td>34</td>
<td>27</td>
</tr>
<tr>
<td>MIC (mg/mL)</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
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</tbody>
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Table 3: The zone of inhibition and MIC (in mm) of Gentamycin against the test organism.
Figure 5: (a) and (b) Inhibition % of S.aureus, (c) and (d) Inhibition % 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 translation 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 urethritis, cystitis, pylonephritis, prostatitis and pneumonia disease, and can be used as new drug for therapy.

ACKNOWLEDGEMENT

The authors acknowledge the facilities provided by the Department of Zoology, Ranchi University, Ranchi.

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