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Comparative study of antimicrobial activity of Andrographis paniculata and ocimum sanctum with commercial antibiotics

Dr. S Subeena Begum
Assistant Professor in Zoology, Department of Animal Health and Management, Alagappa University, Karaikudi. Tamil Nadu, India

Abstract
Methanol extracts obtained from *Andrographis paniculata and Ocimum sanctum* have been investigated for their antimicrobial activity with disc diffusion method. Antimicrobial activity was determined with pathogens such as Gram positive bacteria Staphylococcus aureus and Gram negative bacteria Aeromonas hydrophila, *Escherichia coli*, by the disc diffusion method. The extracts of *Andrographis paniculata and Ocimum sanctum were impregnated with empty sterile disc in the concentration of 6mg/disc, 3mg/disc1.5mg/disc0.75mg/disc.* The extracts of *Andrographis paniculata and Ocimum sanctum* had strong antimicrobial activity against the Gram-positive bacteria but slightly lesser activity was recorded against the Gram-negative bacteria used in this study. Zone of microbial inhibition for gram positive, *Gram negative bacteria* were significantly higher in the extract of Andrographis *paniculata (6mg/disc) than Ocimum sanctum (6mg/disc) and commercially available antibiotic in the concentration of* (10µg).

Keywords: *Andrographis paniculata; Aeromonas hydrophila; Ocimum sanctum; Staphylococcus aureus*; zone of microbial inhibition

1. Introduction
In recent years, multiple resistances in pathogens have developed due to the in discriminate use of antimicrobial drugs such as antibiotics commonly employed in the treatment of infectious diseases. The undesirable side effects of most of the antibiotics and the emergence of previously uncommon infections have forced researchers to investigate the new antimicrobial substances from various sources like medicinal plants. The screening of plant extracts for antimicrobial activity has shown that several medicinal plants represent a potential source of new anti-infective agents (Poole, 2001; Salvat *et al*., 2001; Arias *et al*., 2004) [13]. Some of the medicinal plants such as *Andrographis paniculata and Ocimum* sanctum are rich in antimicrobial components. Their plant extracts can be replaced the usage of antibiotics in eco-friendly way.
Most of the medicinal plants have been used widely throughout centuries to treat internal and external infections. Particularly *Andrographis paniculata and Ocimum sanctum* have so many phytocomponents such as flavanoids, saponin, glycocortecoids, tannin and secondary metabolites which are very effective in antibacterial activity. Many internal and external uses of the leaves of medicinal plants have been documented in many societies in Europe, Asia, Africa, and North America (Meurer Grimes *et al*., 1996). Many herbal plants have been used for the treatment of scabies, tumors, and bacterial infections (Paris & Moyse., *et al* 1976; Hetaher & Hendersoni, 1994) [12].
Alkaloids, phenolic compounds, amino acids, and tannins are present in considerable amount in all of the medicinal plants. Indian Ayurvedic and traditional Chinese systems are playing important roles in designing new medicines from medicinal plants, which are also rich sources of antioxidants (Mastan *et al*. 2013) [10]. Recent estimate shows that about 80% of people in emerging countries still depend on traditional medicine-based largely on various species of medicinal plants for the crucial healthcare.
Medicinal plants have been known to synthesize active secondary metabolites with established potent anti-microbial activities, which formed the basis for their applications in pharmaceuticals, alternative medicines and natural therapies (Hammer *et al*., 1999; Fabricant and Famsworth, 2001). The antimicrobial activities of the medicinal plants used in the present study could be due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and steroids against both Gram-positive and Gram-negative organisms (Baser, 1993; Jouad *et al*., 2001; Anyanwu and Dawet (2005); Koche *et al*., 2010) [2].
There are many investigations on the microbial activity of leaf extract, but these particular selected medicinal plants such as *Andrographis paniculata and Ocimum sanctum* have not been previously investigated. There is no investigation on comparative analysis on selected medicinal plant leaf extract with commercial antibiotics. Therefore, our aim was to compare the antimicrobial effects of plant extracts obtained from medicinal plants against microorganisms with the commercially available antibiotics such as tetracycline and ampicillin.

2. Materials and Methods
Plant materials
Leaves of *Andrographis paniculata and Ocimum sanctum* were collected from Pudukottai district. Voucher specimens of the plants were authenticated by Dr. Jothibasu, Assistant professor, DDE, Alagappa University.

Preparation of extracts
The plants parts were air-dried. Each dry powdered plant material (20 g) was extracted with 150 mL of 80% methanol for 24 h by using Soxhlet equipment (Khan *et al*., 1988) [9]. The extract was filtered using Whatman filter paper no.1,

and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw-capped bottles at −20°C.

Microorganisms

Aeromonas hydrophila, Escherichia coli, Staphylococcus aureus (MTCC) were used as test microorganisms.

Screening for antimicrobial activities

The dried plant extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 200 mg/mL and sterilized by filtration through 0.45-µm membrane filter. Empty sterilized antibiotic discs having a diameter of 6 mm were impregnated with 50 µL of extract (10 mg/disc) at a concentration of 200 mg/mL. All the bacteria mentioned above were incubated at 35 ± 0.1°C for 24 h by inoculation into nutrient broth. An inoculum containing 10^6 bacterial cells was spread on Mueller-Hinton agar plates (1 mL inoculum/plate). The empty sterile discs impregnated with extracts were placed at 4°C for 2 h, bacteria were incubated at 35 ± 0.1°C for 24 h (Collins *et al*., 1989; Collins CH, Lyne PM, Grange JM.,1989; Ali-Stayeh *et al*., 1998). At the end of the period, inhibition zones formed on the medium were evaluated in millimeters. Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disc was applied, depending on the test microorganism for comparison.

3. Results

Table 1: Comparison of Antibacterial activity of *Andrographis paniculata, Ocimum sanctum* with broad spectrum antibiotics against the selective human and fish pathogens. (Data represent the average ± standard deviation from the triplicate).

Concentration of medicinal plant leaf extracts and antibiotics	Human and fish pathogen (*Aeromonas hydrophila)*	Human Pathogen *E. coli*(Gram negative bacteria)	Human Pathogen(*Staphylococcus aureous*)Gram positive bacteria)
Andrographis paniculata 6mg 6mg	13mm ± 0.05	12mm ± 0.06	24mm ± 0.03
3mg	6mm ± 0.05	2mm ± 0.08	12mm ± 0.06
1.5mg	3mm ± 0.04	1mm ± 0.1	12mm ± 0.03
0.75mg	---	---	6mm ± 0.07
Ocimum sanctum 6mg	10mm ± 0.1	10mm ± 0.01	19mm ± 0.09
3mg	7mm ± 0.08	5mm ± 0.04	10mm ± 0.1
1.5mg	2mm ± 0.02	1mm ± 0.06	5mm ± 0.06
0.75mg	--	--	1mm ± 0.09
Tetracycline(10µg)	10mm ± 0.07	10mm ± 0.06	12mm ± 0.1
Ampicillin(10µg)	9mm ± 0.06	9mm ± 0.03	12mm ± 0.04

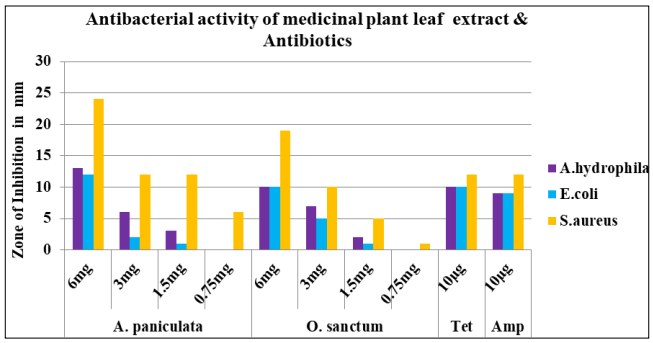

Fig 1: Concentration of medicinal plant leaf extracts and antibiotics

Table.1, shows that the microbial inhibition zone of the plant extracts, the inhibition zones formed by standard antibiotic discs and plant extract impregnated disc.

Significant antimicrobial activity was found against Gram-positive bacteria *Staphylococcus aureus*, and also Gram-negative bacteria such as *Escherichia coli*., and *Aeromonas hydrophila*,. Antimicrobial activity was very effective against the Gram-positive bacteria such as *Staphylococcus aureus* in all the concentrations of extracts of *Andrographis paniculata* and *Ocimum sanctum. Highest Antimicrobial activity was observed in the extract of Andrographis paniculata* than *Ocimum sanctum. The antibiotic discs such as tetracycline and ampicillin showed antimicrobial activity equal to 6mg extract impregnated disc of Ocimum sanctum, Meanwhile zone of microbial inhibition was higher in the 6mg impregnated extract of Ocimum sanctum than in antibiotic disc. However, Andrographis paniculata and Ocimum sanctum disc the showed higher bacterial inhibition zone in positive bacteria Staphylococcus aureus than the gram negative bacteria such as Escherichia coli*., and *Aeromonas hydrophila. In E. coli microbial inhibition zone was higher in 6mg extract of Andrographis paniculata and Ocimum sanctum than in antibiotic discs tetracycline(10µg)and Ampicillin* 10 *µg.*

Fig.1 clearly indicates that extracts of *Andrographis paniculata* have significantly higher antibacterial zone in the gram positive bacteria *Staphylococcus aureus(zone of inhibition 24mm) and* gram negative bacteria such as *Aeromonas hydrophila* and *E. coli zone of inhibition is* (13mm and 12mm) *respectively. it's antimicrobial zone was slightly higher* than the *Ocimum sanctum* and antibiotics. *Staphylococcus aureus* is more susceptible to the extracts of *Andrographis paniculata with the highest inhibition zone of 24mm. This result clearly shows that* the *Andrographis paniculata* showed significant antimicrobial activity against Gram-positive bacteria and also gram negative bacteria. However, There is no microbial inhibition zone in 0.75mg extract of *Ocimum sanctum Andrographis paniculata* against gram negative bacteria but there is a microbial inhibition zone of 1mm was measured in gram positive bacteria *Staphylococcus aureus.*

In general, the result shows that Gram-negative bacteria have been found to be more resistant to extracts of both *Ocimum sanctum and Andrographis paniculata* than Gram-positive bacteria, in very low concentration. *Staphylococcus aureus* was more susceptible to the extracts of both plants.

4. Discussion

It was found that extracts of *Andrographis paniculata* have significantly higher antibacterial zone in the gram positive

bacteria *Staphylococcus aureus the zone of inhibition is 24mm and* gram negative bacteria such as *Aeromonas hydrophila* and *E.coli zone of inhibition is* (13mm and 12mm) *respectively. However the zone of inhibition was slightly highe*r than the *Ocimum sanctum* and antibiotics. *the same report is documented in* the leaf extracts of *Scrophularia* were found to be effective against Gram-positive bacteria,(Basaran Dulger 2008); in ethanol extract of *Andrographis paniculata* zone of inhibition is 19.4 in *E. coli* (Neha Sinha., 2016) [11]; The fresh methanol leaf extract exhibited the maximum inhibitory activity against the gram positive bacteria and slightly lesser inhibitory activity against in gram negative bacteria(Neha Sinha., 2016) [11]; Zone of inhibition (mm.) of human pathogens Staphylococcus sp in ethanol extract 9.2mm The activity of ethanolic leaf extracts showed maximum zone of inhibition (25.2 mm) for Salmonella typhi and the minimum zone of inhibition (9.2 mm) for Staphylococcus sp (Arunadevi *et al* 2010).The extract showed significant antimicrobial activity, which may be due to the effect of the arabinogalactan and andrographolide present in *Andrographis paniculata*, (Prajall singa *et al*., 2004). Andrographispaniculata plant extract is known to possess a variety of pharmacological activities. Andrographolide, the major constituent of the extract, is implicated in its pharmacological activity (Neha Sinha., 2016) [11]

It was found that extracts of *Ocimum sanctum* have significantly higher antibacterial zone in the gram positive bacteria *Staphylococcus aureus* the zone of inhibition is *19mm and* gram negative bacteria such as *Aeromonas hydrophila* and *E. coli* zone of inhibition was 10mm. it's antimicrobial zone was same in 3mg extract of *Ocimum sanctum* and 10µg of antibiotics. The extract showed significant inhibition of bacterial zone, which may be due to the effect of the alkaloid, glycoside, terpenoid, steroid, flavonoid, tannins, eugenol and methyl eugenol. Similar findings were well reported; when ethanolic extract of *Ocimum sanctum* against *aggregatibacter actinomycetemcomitans*, *prevotella intermedia*, and *porphyromonas gingivalis*. Sajjanshetty Mallikarjun *et al*., 2016

When extract of *Ocimum sanctum* studied against *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus the zone of inhibition* were 16 mm, 14 mm, and 17 mm, respectively (Saurabh and Komal., 2017) [17]; O. sanctum extract against Streptococcus mutan Agarwal *et al.*, 2016 [1]; Similar studies on antimicrobial properties of the extract of *Ocimum sanctum* against *S.aureus* and enteric bacteria., (Vasudevan *et al*., 2001) [19]. Similar report has been well documented against strains of *Pseudomonas aeruginosa*, *Shigella sp.*, *Listeria monocytogenes*, *Staphylococcus aureus* and two different strains of *Escherichia coli* in the the methanol extracts *Ocimum Basilicum* L(Ilhan Kaya, *et al*.,2008). In the present study, *Andrographis paniculata, O. sanctum* extracts inhibited the growth of *Aeromonas hydrophila*, *E. coli* and *Staphylococcus aureus.* It's zone of inhibition in the concentration of 6mg extracts of both were observed higher than in antibiotic disc of tetracycline and ampicillin.

These results are broadly similar to those of studies that used disk diffusion method, Similar reports are well documented toward Gram positive and Gram negative bacteria, (Mahmood *et al*., 2008; Helen *et al.*, 2011; Mishra and Mishra, 2011; Mahmood *et al*, 2008) [3, 7, 14]. Similarly Mishra and Mishra, (2011) reported good inhibition of both Gram-positive and Gram-negative species.

Among selected plant extracts the zone of inhibition was highest in *Andrographis paniculata* than *Ocimum sanctum*. The inhibition zone were recorded in selected broad spectrum antibiotics against gram positive and gram negative bacteria. Area of zone of inhibition was slightly lesser in gram negative bacteria than in gram positive because of their cell wall lipopolysaccharide (Farbood *et al.*, 1976) [5]

Infectious diseases are a major cause of morbidity and mortality worldwide. Currently, the ongoing battle against bacteria prevails certainty of evolving, resistance. On the other hand, advancement in medical field results in more patients being in critical and immune suppressed states, thus creating a perpetual need for new antibiotics. As a result, it is the right time to discover new antibiotics (Mahesh and Satish, 2008).Medicinal plants are more important in field of pharmaceutical industries for new drug preparation (Sule *et al*., 2010) [18].

5. Conclusion

Both selected extracts produced good inhibition zone against the gram positive and gram negative bacterias. As it is eco-friendly it could be used to treat infections and disease caused by those microorganisms instead of commercially available antibiotics. However active ingredients of the extracts must be purified, isolated, characterized and field evaluation are needed.

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7. References

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