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In-vitro antibacterial study of *Aquilaria agallocha* heart wood oil and *Citrullus lanatus* seed oil

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Abstract – The antibacterial activity of *Aquilaria agallocha* heart wood oil and *Citrullus lanatus* seed oil were screened by using agar well diffusion method taking organisms *Escherichia coli*, *Staphylococcus* aureus, *Pseudomonas* aeruginosa, *Enterococcus faecalis.* Different concentrations of (2.5% (V/V), 5% (V/V) and 10% (V/V)) of each oil were made in DMSO and the zone of inhibition was calculated for each concentration and compared with standard ciprofloxacin (10 mcg/ml). From this study it was found that both oil posses the anti-bacterial activity. But *Citrullus lanatus* seed oil shows more anti-bacterial activity than *Aquilaria agallocha* oil.

Keywords – *Aquilaria agallocha*, CCl_4induced liver damage, serum enzyme levels, Histopathology.

INTRODUCTION

Medicinal plants can able to protect from different deadly microbial species. These plants are used in different forms under indigenous systems of medicine like Ayurveda, Sidha and Unani [1]. Rapid and overdose of synthetic tic drugs can cause resistance development in the body [2]. So, in comparison to synthetic drugs herbal medicines are safer [3]. According to WHO, 80% of the world's inhabitants problem should treated by medicinal herbal drug for their primary health care [1].

Agar wood (*Aquilaria agallocha* of family Thymelaeaceae) oil is extremely rare and precious oil available in North Eastern India, Bhutan and parts of South East Asia. The different extracts of the plant has reported to possess anti nociceptive [3], anti-microbial [4], lower hypersensitivity reactions [5], laxative [6], anti oxidant activity [7], CNS activity [8], sedative effect [9] and anti-hyperglycaemic activity [10].

Citrullus lanatus of family Cucurbitaceae is commonly known as water melon and in local name Tarmuz (Hindi), Puchakaya (Telugu). The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. Fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain [11]. It also reported having analgesic and anti-inflammatory activity of roots and leaves [12], antimicrobial activity [13], laxative activity of fruit [14], anti-oxidant and antiulcerative activity [15].

According to literature, fruit extract of *Citrullus lanatus* and leaves extract of *Aquilaria agallocha* having anti-microbial activity. But no literature available for antimicrobial activity of seed oil *Citrullus lanatus* and heartwood oil of *Aquilaria agallocha.* Therefore, the present study was designed to evaluate *in-vitro* antimicrobial activity of *Aquilaria agallocha and Citrullus lanatus* seed oil.

MATERIALS AND METHODS

Plant material

The seeds of *Citrullus lanatus* of family Cucurbitaceae were collected from ripe fruits which were obtained from local fruit market, Kodad, Andhra Pradesh. The seeds collected from fruit and dried and extracted with n-hexane to obtain the oil. The percentage yield was 21.59 % w/w.

The *Aquilaria agallocha* oil was obtained as hydro distillation from heart woods of plants and obtained as sample from Vendor from Hojai, Assam.

Drugs and chemical

The standard drug ciprofloxacin was used for this study and purchased from retail pharmacy shop. n-Hexane used in the extraction of seed oil was of analytical grade.

In-vitro antibacterial activity (Well-diffusion method)

The antibacterial activities of *Aquilaria agallocha* oil *and Citrullus lanatus* oil were determined by agar well diffusion method [16]. Briefly, pure isolate of each bacterium was first sub-cultured in nutrient broth at 37ºC for 24h. One hundred microlitres (100µL) of the standardized inoculum (106CFU/mL; 0.5 Mac-Farland) of each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton Agar plate (Hi Media) so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0mm was used to bore wells in the agar plates. Subsequently, a 50µL volume of the the oil was introduced in triplicate wells into Muller-Hinton Agar plate. Sterile DMSO served as negative control. A positive control in the form of Ciprofloxacin (10 mcg/ml) was also included in the study. The plates were allowed to stand for atleast 1h for diffusion to take place and then incubated at 37ºC for 24h. The zone of inhibition was recorded to the nearest size in mm. **[17]**. Test organisms used were *Escherichia coli*, *Staphylococcus* aureus, *Pseudomonas* aeruginosa, *Enterococcus faecalis*. Different concentrations (2.5% (V/V), 5% (V/V) and 10% (V/V)) of each oil were made in DMSO and the zone of inhibition was calculated for each concentration.

RESULTS AND DISCUSSION

Four varieties of bacteria were tested for their sensitivity *Aquilaria agallocha* oil *and Citrullus lanatus* oil. The antibacterial potency was determined by the well diffusion method and the result is given in **Table-1**. The result presents diameters of inhibition zones (clear zones around wells) exerted by the various concentrations of tested drugs and standard ciprofloxacin. Between these two plants *Citrullus lanatus* oil showed more anti-bacterial activity than the other. Both the plants oil contains phenolic compounds and triterpinoids [16, 17]. Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes. Actually, phenolics not only attacked cell wall and cell membrane, thereby destroying its permeability and releasing of intracellular constituents (ribose, Na glutamate, etc.) but also interfered with membrane function e.g. electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity. That was, active compounds might have several invasive targets which could lead to inhibit the bacteria. Furthermore, leakage of intracellular material was a general phenomenon induced by many antibacterial substances.

Table 1: Antibacterial activity of *Aquilaria agallocha* oil *and Citrullus lanatus* oil Mueller- Hinton agar medium

Sl. No.	***Bacteria***	***Aquilaria agallocha oil* (Zone of Inhibition in mm diameter)**			***Citrullus lanatus* oil *oil* (Zone of Inhibition in mm diameter)**			***Positive Control (Ciprofloxacin 10 mcg/ml)***	***Negative control DMSO***
		2.5% (V/V)	**5% (V/V)**	**10% (V/V)**	**2.5% (V/V)**	**5% (V/V)**	**10% (V/V)**	**35.6 ± 0.27**	**-**
1	*Escherichia coli*	7.1 ± 0.12	8.4 ± 0.15	9.5 ± 0.13	16.8 ± 0.12	19.6± 0.13	25..5 ± 0.14	31.6 ± 0.17	0
2	*Staphylococcus* aureus	6.8 ± 0.07	7.4 ± 0.10	7.9 ± 0.08	14.4 ± 0.16	18.0± 1.22	24.3 ± 0.16	20.5 ± 0.21	0
3	*Pseudomonas* aeruginosa	8.1 ± 0.13	8.2 ± 0.13	8.7 ± 0.11	11.4 ± 0.15	13.9 ± 0.12	25.5 ± 0.19	39.3 ± 0.30	0
4	Enterococcus *faecalis*	5.3 ± 0.14	6.3 ± 0.11	7.1± 0.22	14..5 ± 0.13	22.3 ± 0.16	23.5 ± 0.24	32.6 ± 0.22	0

Incubation temperature: 37ºC; **Incubation period:** 24h, **Negative control**- Dimethyl sulfoxide; **Positive control**- Ciprofloxacin, LµVolume oil in each well = 50 ; (Values are given as Mean± SD of three independent replicates)

CONCLUSION

From this study it can be concluded that both oil posses the anti-bacterial activity. But *Citrullus lanatus* seed oil shows more anti-bacterial activity than *Aquilaria agallocha* oil.

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