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Statistical analysis of the antibacterial activity of *Zanthoxylum rhetsa* seed essential oil

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ABSTRACT

Antibacterial activity of Zanthoxylum rhetsa seed essential oils steam distilled from aqueous, alkaline and acidic media against four pathogenic bacteria namely Staphylococcus aureus, Escherichia coli, Proteus vulgaris and Klebsiella pneumoniae were statistically evaluated. The acid distilled oil showed significant activity against all the tested microorganisms, and its activity is quite comparable with the standard antibiotic gentamicin screened under similar conditions. So, the acid distilled seed essential oil of Zanthoxylum rhetsa can be used as a potential external antiseptic and can be incorporated into drug formulations. The activity of aqueous and alkaline extract was found to be moderate. Zanthoxylum rhetsa seed oil showed significant activity against these organisms probably due to the synergic effect of the components present in the oil.

Key words: *Zanthoxylum rhetsa*, seed essential oil, antibacterial activity, statistical analysis, drug formulation.

INTRODUCTION

Zanthoxylum rhetsa (Rutaceae family) is a lofty, deciduous tree, up to 35 m. tall, commonly found in the evergreen monsoon forests of the foothills of Assam and Meghalaya and in the eastern and western ghats in peninsular India. The fruits yield an essential oil called Mullilam oil, which is obtained by steam distillation of the dried ripe fruits. The oil has pleasant odour resembling that of sweet orange and tangerine. It is used in the indigenous system of medicine

for the treatment of cholera [1]. The oil is also used as an antiseptic, a disinfectant, and for the treatment of asthma, toothache and rheumatism [2].

Earlier works [3-7] revealed that the seed oil was rich in monoterpenes, especially sabinene; while caryophyllene oxide was the major component of the leaf oil. Paknikar and Kamat [8] isolated a crystalline compound from *Z. rhetsa* fruit oil and assigned its structure as 1S, 2S, 4S-trihydroxy-p-menthane. The dried, ripe seeds of *Zanthoxylum rhetsa*, on steam distillation yielded an essential oil. The oil is used as an antiseptic and disinfectant [1-2].

In the present study antibacterial activities of the seed essential oils of *Z. rhetsa* steam distilled from aqueous, alkaline and acid media were analyzed statistically so as to evaluate the medicinal potential of the seed essential oil extracted from media having different pH.

EXPERIMENTAL SECTION

Plant Material

Fresh seeds of *Zanthoxylum rhetsa* (Rutaceae) were collected in September from Kerala, South India and authenticated by Dr. A.K. Pradeep, Dept. of Botany, Calicut University. Voucher specimen (No.50) is deposited in the specially maintained herbarium, Department of Chemistry, Calicut University.

Essential oil extraction

The fresh greenish black seeds of *Z. rhetsa* (300g each) were steam distilled from water (500 ml), alkaline solution (500 ml of 10% Na₂CO₃), and acid solution (500 ml of 4% H₂SO₄) for 3h., without powdering or maceration. The distillate was collected in each case, extracted with diethyl ether (2x 100 ml) and dried over anhydrous sodium sulphate. The solvent on evaporation yielded 5.5g (1.83%), 4.5g (1.5%) and 3.26 g (1.08%) of oil, based on fresh weight of the seed [9].

Table 1: Student's t-test: Mean activities of *Z. rhetsa* seed essential oil steam distilled from aqueous, alkaline and acid media

<i>Z. rhetsa</i> seed essential oil steam distilled from aqueous, alkaline and acid media	Calculated value of 't'	Correlation coefficient
aqu100%- alk100%	0.337	0.9321
aqu100%- acid100%	-2.247	0.1782
alk100%- acid100%	-2.271	0.2977
aqu10%- alk10%	0.721	0.9590
aqu10%- acid10%	-1.525	0.8921
alk10%- acid10%	-1.845	0.9814
aqu5%- alk5%	0.656	-0.2927
aqu5%- acid5%	-5.461	-0.4402
alk5%- acid5%	-5.095	0.9537
aqu1%- alk1%	1.011	-0.0909
aqu1%- acid1%	-1.831	0.6625
alk1%- acid1%	-2.211	0.2548

aqu: *Z. rhetsa* seed essential oil steam distilled from aqueous medium

alk: *Z. rhetsa* seed essential oil steam distilled from alkaline medium

acid: *Z. rhetsa* seed essential oil steam distilled from acidic medium

Antibacterial activity

Zanthoxylum rhetsa seed essential oil was examined for antibacterial activity [10] against Gram-positive bacterium, *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*. Gentamicin, inhibiting bacterial cell wall biosynthesis was used as standard for comparison. The agar diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8 cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50 μ l of *Z. rhetsa* seed essential oil in DMSO (Dimethyl sulphoxide) were added in each well. The diameter of the inhibition zone was measured in mm.

Statistical analysis

By applying t-test, the mean activity of *Z. rhetsa* seed essential oil steam distilled from aqueous, alkaline and acid media at various concentrations (100%, 10%, 5% and 1%) are compared (Table 1). The mean activity of acid 100% > aqu 100%, acid 100% > alk 100% (activity of 'acid 100%' greater than 'alk 100%'), acid 10% > alk 10%, acid 5% > aqu 5%, acid 5% > alk 5%, acid 1% > aqu 1%, acid 1% > alk 1% while that of aqu 100%-alk 100%, aqu 10%-alk 10%, acid 10%-aqu 10%, aqu 5%-alk 5%, and aqu 1%-alk 1% are almost same.

The interval estimation of the mean activity of *Z. rhetsa* seed essential oil steam distilled from aqueous, alkaline and acid media are given in table 2.

Table 2: Interval estimation of the mean antibacterial activity of *Z. rhetsa* seed essential oil steam distilled from aqueous, alkaline and acid media

<i>Z. rhetsa</i> seed essential oil steam distilled from aqueous, alkaline and acid media	Mean activity	Standard Deviation	Interval estimation at 5% level of significance
aqu 100%	27.75	3.593	27.75 \pm 3.52
aqu 10%	18.75	3.095	18.75 \pm 3.03
aqu 5%	13.5	1.00	13.5 \pm 0.98
aqu 1%	11.75	0.957	11.75 \pm 0.94
alk 100%	26.5	5.323	26.5 \pm 5.21
alk 10%	16.5	4.434	16.5 \pm 4.34
alk 5%	12.75	1.707	12.75 \pm 1.67
alk 1%	10.5	1.915	10.5 \pm 1.87
acid 100%	38.75	7.675	38.75 \pm 7.52
acid 10%	26.75	8.539	26.75 \pm 8.36
acid 5%	20.25	1.893	20.25 \pm 1.85
acid 1%	15.5	3.416	15.5 \pm 3.34
gen 200 μ g	26.25	5.252	26.25 \pm 5.14

gen: Antibiotic gentamicin

The activity of *Z. rhetsa* seed essential oil steam distilled from aqueous, alkaline and acidic media (100%) and the antibiotic gentamicin (200 μ g) against four pathogenic bacteria are shown in fig 1.

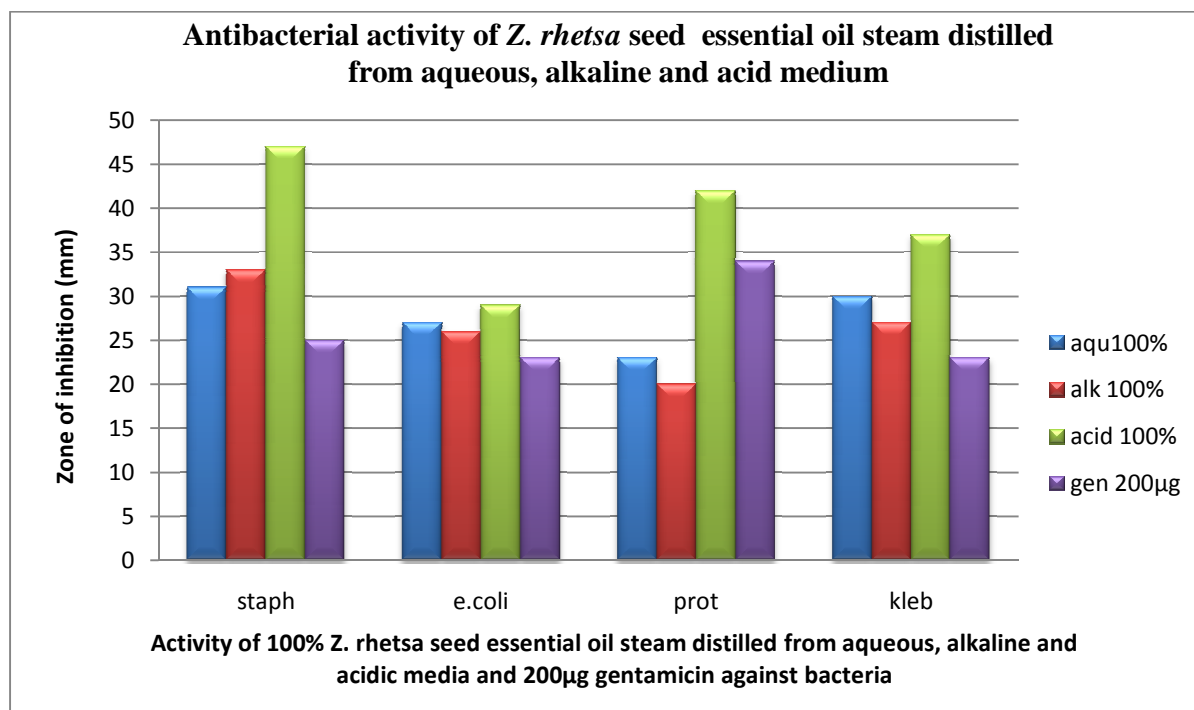


Fig. 1: Antibacterial activity of *Z. rhetsa* seed essential oil steam distilled from aqueous, alkaline and acidic media and gentamicin against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

(**gen:** Antibiotic gentamicin; **staph:** *Staphylococcus aureus*; **e. coli:** *Escherichia coli*; **prot:** *Proteus vulgaris*; **kleb:** *Klebsiella pneumoniae*)

RESULTS AND DISCUSSION

The correlation studies suggests that aqu100%-alk100%, aqu10%-alk10%, alk10%-acid10% and alk5%-acid5% are highly correlated and hence the mean activity of these oil samples against four pathogenic bacteria increases simultaneously whereas aqu5%-alk5%, aqu5%-acid5%, aqu1%-alk1% and aqu100%-aqu5% are negatively correlated and the activity of these oil samples against the bacteria changes in an inverse manner.

Aqu10%-acid10%, aqu1%-acid1% showed appreciable positive correlation though not high, whereas aqu100%-acid100%, alk100%-acid100% and alk1%-acid1% showed low positive correlation,

The activity of *Z. rhetsa* seed essential oil steam distilled from acid medium at various concentrations (100%, 10%, 5% and 1%) and activity of standard antibiotic gentamicin (200µg) are statistically analysed and found that the activity of 5µl of 100% oil sample is higher than 200µg gentamicin. The activity of 10% acid distilled oil sample against four pathogenic bacteria was found to be same as that of 200µg gentamicin, whereas the activity of 5% seed oil was quite comparable with the standard antibiotic. The acid distilled oil sample at concentrations 100%, 10% and 5% showed appreciable positive correlation with that of the standard antibiotic gentamicin (200µg). Hence *Z. rhetsa* seed essential oil steam distilled from acid medium can be used as a potential antibiotic and can be incorporated into the drug formulations.

Statistical analysis of antibacterial activity of the *Z. rhetsa* seed essential oil steam distilled from aqueous, alkaline and acidic media revealed that, the acid medium extract exhibited pronounced activity towards the tested microorganisms. The activity of the *Z. rhetsa* seed essential oils differ considerably with the microorganisms used. All the oil samples showed only moderate activity towards the Gram-negative bacteria *Escherichia coli*. Both alkaline and aqueous medium extract showed moderate activity against all the tested microorganisms, at all concentrations and their results were quite comparable. The *Z. rhetsa* seed essential oil obtained by steam distillation from acid medium, was found to be highly active against all the tested bacteria especially with *Staphylococcus aureus*, *Proteus vulgaris* and *Klebsiella pneumoniae* at a concentration of 100%. Significant activity was also shown by the acid medium extract at a concentration of 10%, and 5%.

The GC-MS analysis of the *Z. rhetsa* seed essential oil obtained by steam distillation from aqueous, alkaline and acid media showed that α -terpinene, γ -terpinene and terpinolene are the main constituents present in the acid medium extract, while sabinene, β -pinene, α -pinene and myrcene were the major components obtained from aqueous and alkaline media. Terpinenes and terpinene derivatives are reported to have antibacterial activities [11]. α - and γ -terpinenes and terpinolene are the chief constituents of acid extract and the antibacterial activity of the oil can be attributed to the presence of these components.

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