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ESSENTIAL OIL OF ROSE CENTIFOLIA L. FLOWER SHOW AN ANTIMICROBIAL ACTIVITY

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ABSTRACT
Rose centifolia L. Oil revealed wide spectrum of antibacterial antifungal properties against some pathogen including Bacillus Staphylococcus aureus, Pseudomonas aeruginosa (Eris R.Ulusoy2013). Essential oil of Rosa centifolia show various pharmacological properties have been attributed in preparation of Attars, Body perfumed, Body massage oil and Hair oil, which is in therapeutic effect on body (Zargari et.al1992). Thus a very basic and practical study is required. The n-hexane flower extract of Rosa centifolia L. oil was prepared and analyses for its antimicrobial activity. A low effect was found against Staphylococcus auresus, a gram positive test organism by susceptibility disc method. 25% inhibition was found against staphylococcus aureus by the Durham's diffusion tube indicating antimicrobial properties with lower inhabitance in plant flower.

KEYWORDS: Rosa centifolia L., N-Hexane, Antimicrobial, Staphylococcus aureus.

INTRODUCTION

Some Historical evidence show that Rose oil is originated from Greece. Rose oil is the essential oil extracted from the Patel of Rose centifolia L.(Zargari et.al.1992).

Most species of flower have essential oil in addition to their antioxidant properties they posses antibacterial and antifungal activity. The flower contain camphor 41%, germacrene 16%, transpinocarveol 11% which were found to possess inhibitory activity against Gram positive bacteria and some Fungi (Juteae et, al. 2002).

Rose are the most popular and widely used medicinal plant all over the world. Rose plant originated from middle east and cultivated all over the world. (Krussman1981).

The most important component of Rose oil is Terpene, Glycosides, Flavonoids, Anthocyanin and n-Hexane (Almasirad et.al.2007, Schiber et.al. 2005).

Pharmacological activities of Rose oil evaluated by several in vitro and vivo (Maleev et.al.1972).

Essential Rose oil has been administered as aromatherapy so it demonstrated that Rose oil can be absorbed in to the body via the skin or the olfactory system (Dye 1997).

Many studies view that olfactory stimulation by Rose oil could produced instant change in physiological parameter including muscle tension, blood presser, pulse rate, skin temperature, electro dermal activity and brain activity(Digo et. al.1998).Rose oil also showed beneficial effect in reducing menstrual pain and bleeding (Marzule et.al.2013).

The essential oil contain various component which impart the antimicrobial activity. Compound such as methyl pyrimidine, beta glucopyranosyltriene isolate from flower showed the antifungal and antibacterial activity against many gram positive and gram negative organisms.(Arjun et.al.2002). Such a compound has been investigated by the well agar diffusion medium method.

There is a great scope for the use of essential oil in various prepossess. The study of Essential oil of Rose centifolia L. flower show an antimicrobial activity, reveals that, many component explored in essential oil but to do a best exploration, a basic and practical study is required, the present studies are aim that the qualitative analysis on the antimicrobial studies of the extracted essential oil by flower petale,used Disc susceptibility method and Durham fusion tube method (for volatile compound).

MATERIALS AND METHOD

The fresh Rose centifolia L. Flower were collected and then shade air dried. The essential oil were extracted using the solvent extraction method (Guenther 1972).

Nutrient agar media was used for maintaining standard test microorganism and subculture. Muller Hinton Agar was used for the actual disc susceptibility test. Nutrient Broth and Tripticase Soya Broth (TSB) were used for preparation of the inoculum densities. For the antimicrobial activity four test organism include two gram positive and two gram negative strain. The four microorganism like Staphylococcusaureus ATCC25923, Staphylococcus epidermis, Pseudomonas aeruginosa and Escherichia coli ATC1014Bisolated from standard nutrient agar media at 37 Incubation temperature.

Susceptibility Disc Method; The Antimicrobial essay carried out by method **(**Jacques 1980).

Collection and maintenance of culture; The cultures were preserved at 4^0C in refrigerators after 24 hr inoculation. The culture were subculture every week to maintain their purity and viability.

Preparation of inoculum; few of colony were selected from the master plates and suspended in a small volume of 5ml saline. The inoculation can also be prepared in TSB medium. The inoculum was incubated for 1-2 hour and terbidity of culture was matched with that of I standard of MC Farland opacity tube the turbidity was adjusted with the help of saline, TSB. (mcFarland turbidity standard of I is equal to inoculum of 3 $*10^8$ cells/ml.).

Inoculation of nutrient agar plate; The entire surface of plate was inoculated and 0.1 ml of the adjusted culture was added to the plates with the help of sterile pipettes. A sterile cotton swab was rotated several time in all direction to dry the inoculum on the plate and thus ensure even distribution and diffusion of the culture.

Preparation of Antimicrobial discs; In accordance to FDA Standard sterile plain filter paper discs (SD067) used. 20ul Rose centifolia L. essential oil extract was carefully added on the discs with the help of sterile pipette. The discs were stored at -20^0C for 30 minutes and then used for inoculation. The same procedure used for nHexane to check for its antimicrobial activity.

Application of discs; 10-15 minutes after inoculation of plate the Rose essential oil and nHexane discs placed in center of each inoculated plate. The result were observed 18-24 hrs after the inoculation and incubation.

Durham's Diffusion Method; For volatile essential oil to study the antimicrobial properties of Rose centifolia L. (Agnihotri and Vaidya, 1996).

Collection and maintenance of standard test culture, Preparation of inoculum is same as to Susceptibility Discs test.

Inoculation of Nutrient agar; Nutrient agar slant were incubated by streaking a loopful of the prepared inoculum and incubating at 37^0C for 24 hrs.

Preparation of Durham's tubes; Autoclaved Durham's fusion tubes (2mm diameter) were picked up by sterile forceps and 0.1 ml of the Rose essential oil extract added to it. Control tube with same quantity of nHexane are also filled and were used as positive control. These tube introduced as inoculated cultural tube or slants. The tube then incubated at 30^0 angle let the emerging vapours from the essential oil extract and Hexane act on the inoculated slants. The result were read after 24 hrs and the percentage of inhibition was recorded.

Interpretation of the Result; The inhibition of bacterial growth noted in the form of total slants expressed. The bacterial growth restricted only to the proximal end of the fusion tube and approximately covering 25% of the slants area was designated as (+). No inhibition of organism from proximal to distal end of tube was designated as (-).

Table 1: Effect of rose essential oil extract and nHexane on the test organisum(Durham's Diffusion Method).

Microorganisum	**Essential oil tube (24hrs)**	nHexane Control tube (24hrs)
Staphylococcus aureus ATCC25923	–	–
Staphylococcus epidermis	_	_
Pseudomonas aeruginosa	+	_
Escherichia coli ATC10148	_	_

RESULT AND DISCUSSION

The result carried out from the primary qualitative analysis of nHexane extract for its antimicrobial activity by Durham diffusion method from freshly prepared essential oil of Rose centifolia L. are shown in table 1; it is found 25% inhibition in Pseudomonasaeruginosa result that 25% of growth on slant at mouth of Durham tube were inhibited by essential oil extract. No such inhibition found in case of other three microorganism. Also inhibition of nHexanecan notseen to any of the four test microorganism. Later essential oil extract and the hexane had completely volatilized after 24 hours.

Table 2: Effect of rose essential oil extract Disc and nHexane Disc on the test organism (Disc Diffusion Method inhibition obtain in mm).

Microorganisum	Essential oil tube (24hrs)	nHexane Control tube (24hrs)
Staphylococcus aureus ATCC25923	–	–
Staphylococcus epidermis	**14**	_
Pseudomonas aeruginosa	**16**	_
Escherichia coli ATC10148	_	_

From table 2; standered disc diffusion method obtained against four test microorganism. Result found with in 24 hours. the hexane disc are put as control can not reach up to inhibition against four microorganism. The solvent does not found any antibacterial activity. From the data of table we observed that the essential oil show an inhibitory zone diameter 16mm against Pseudomonas and very low activity found against Staphylococcus epidermis zone of 14mm diameter. No activity was found against the other microorganisum.

CONCLUSION

The most bioactive compound found in plant of Rose centifolia L. such as Alcohol, Alkaloids show the antimicrobial activity. But in flower essential oil of Rose centifoliaL, bioactive Alcohol absent and thus none of the other compound possess effective for antimicrobial activity. Because alkaloids Liriodenine present reported having anti microbial activity (Khan et.al. 2002).

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