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ORIGINAL RESEARCH ARTICLE

"Antimicrobial Activity of Rose petals Extract Against Some Pathogenic Bacteria"

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ABSTRACT

Rose has influenced cultures aesthetically, economically, medically, religiously and spiritually since humankind could smell and appreciate its fragrance. The high concentration of anthocyanins in the petals gives credence to these indications. They are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agents. The intention of this study was to evaluate the profile of antibacterial activities in rose petals. Result data showed that low dilution of alcoholic extract showed higher antimicrobial activity as compare to high dilution. Petroleum ether extract showed maximum 29 mm zone of inhibition for *Pseudomonas aeruginosa* as compare to other bacterial strains. Alcoholic extract showed higher inhibitory effect on *Streptococcus pneumoniae* (30mm), *Enterobacter aerogens* 28 mm, *Staphylococcus epidermidis* 25 mm, *Bacillus subtilis* 30 mm, *Pseudomonas Aeruginosa* 32 mm. aqueous extract showed higher inhibition against *E.coli* (21 mm), *Enterobacter aerogens (*25mm) and *Bacillus subtilis* (28 mm) as compare to other bacterial strains. Analysis showed that when comparing the antimicrobial activity of Rose petals with control antibiotic, the zone of inhibition was higher to antibiotic at the highest concentration, in the case of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Study showed that the average relative antimicrobial activity was found higher with alcoholic extract (25mm) as compare to aqueous (19mm) and petroleum ether (18mm). Statistical data analysis showed that, petroleum ether showed low average relative antimicrobial activity among all solvents. The finding of antibacterial activity in rose petals in this study indicates its use of petals of rose flowers to cure diarrhoeal diseases, opportunistic infection and skin infection.

(Key word)- Antibacterial activity rose petals, anthocyanins, infectious agents)

INTRODUCTION

Rose has influenced cultures aesthetically, economically, medically, religiously and spiritually since humankind could smell and appreciate its fragrance. The high concentration of anthocyanins in the petals give credence to these indications because anthocyanins are known for their ability to strengthen the vascular system, prevent blood platelet stickiness and also have powerful antioxidant, antibacterial and antinflammatory activity. Rose petal water can also be used as an eyewash and mouthwash (Akhmadieva *et al* 1993).

Rose petal tea is also recommended for treating breast pain or mastitis (a condition often stemming from inflammation and bacterial infection of milk ducts), menstrual difficulties, and the brew are also recommended to soothe a restless fetus (Foster and Duke. 1990). Research has shown that an extract from Rosa canina L. petals (rose red) strikingly increases the effectiveness of several antibiotics against methicillin-resistant Staphylococcus aureus (Rossnagel and Willich. 2001). Other studies have demonstrated strong activity of Rosa canina extract against strains of Candida albicans isolated from clinical samples obtained in the course of acute vaginitis. Additionally, the effect of an anthocyanin preparation isolated from the flower petals of Rosa canina was studied in Chinese hamster fibroblasts and Vicia faba seedlings in respect to cytogenetic damage and mouse survival and there were pronounced radioprotective effects

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demonstrated without any toxic effects (Trovato *et al* 2000).

Rose Petals contains Anthocyanins and proanthocyanidins; tellimagrandin I and rugosin B; carotenoids; plant acids; and essential oils. The liquid portion of oil of rose contains as its chief constituent the alcohol geraniol. Geraniol, with a rose-like odor, is a primary alcohol, and yields, upon oxidation, the aldehyde citral, also present in rose petals. Oil of rose furthermore contains about 20 per cent of l-citronellol. Both geraniol and citronellol are, for the smaller part, combined in the form of ester (about 3 per cent) (Akhmadieva *et al* 1993).

The flower has sepals, petals, androecium and gynoecium a its major organs. The petals of the flowers are variously shaped, coloured and aromatic to attract pollinating insects (Alamaghoul et al 1985). They enclose the androecium and gynoecium and facilitate pollination. The petal tissues, some or all of these may possess antibacterial activity as a natural protection system for reproduction and further perpetuation through seed formation. The Damask rose (*Rosa damascena*), Provence rose (*Rosa Gallica*) and Eglantine(*Rosa elganteraia*) are the three oldest roses in cultivation and are considered to be the most fragrant roses in the world. Rose oil is one of the oldest and best known of all the essential oils. The fragrance of rose is associated with love. It is warm, intense, immensely rich and rosy. It is used in perfumes to lend beauty and depth. A drop or two in a massage, facial or bath oil is luxurious and soothing (Bhakuni *et al* 1988, and Ambastha 1986). The rose flower has five petals (or multiples of five) with numerous stamens, and often exhibits thorny stems or branches. Members of the Rosaceae family grow around the world. There are more than 10,000 types of cultivated rose species, including the popular and fragrant *Rosa centifolia, R. damascena, R. muscatta, R. gallica, R. rugosa, R. indica and R. rubiginosa.* The two main species used in the commercial production of rose oil are *R. centifolia* (Cabbage rose, often hybridized with *R. gallica* to produce Rose de Mai) and *R. damascena*, selected by a majority of perfumers as the standard for floral odors (Özkan *et al* 2004).

A traditional preparation using the rose is rose petal jam, made by cooking rose petals and sugar. Eaten on its own, or made into a drink with milk or fresh yogurt and water, rose petal jam has a cooling effect on mind and body. Rose is perhaps the most celebrated of all fragrances. It is thought to have originated in central Asia, and is mentioned in ancient medical texts from China, India, Persia, Assyria, Egypt, Greece and Rome. In recent years, numerous studies have been also published on the antioxidant, antibacterial activities and chemical composition of rose essential oil (Basim and Basim 2003).

An extract of *Rosa canina* exhibited superior lipid-reducing qualities when compared with eight other natural polyphenolic extracts in vitro. All extracts studied scavenged superoxide radicals and inhibited lipid peroxidation. Researchers summarized that these activities are related to the total polyphenol content of the fruits. A Russian study showed rose oil reduced blood lipid concentrations in rats (Arıdoğan *et al* 2002).

Rose water has a pleasant and refreshing aroma, and its abundance of natural acids makes it an excellent hydrating and anti-inflammatory remedy for skin care. It has disinfecting and soothing properties for dry, delicate and mature complexions and makes a good eye compress for dry, inflamed eye conditions (Gochev *et al* 2008). Whether its properties were discovered through contact with the ethereal realms or through the clinical outcome of the scientific study of its chemical constituents, rose has much to offer on physical, psychological and spiritual levels.

The realization that many infectious pathogenic organisms are fast developing resistance(s) against the prevailing drugs has necessitated a search for new sources of antibacterial compounds. In the course of their life cycle, plants encounter infection by a variety of viruses, bacteria, fungi and parasites specific to them. They are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agent(s) (Williams *et al* 1989). That's why study was aimed to analyze the antibacterial activity of extract obtained from rose patels. The intention of this study was to evaluate the profile of antibacterial activities in rose petals.

Material and Methods

The samples of flower petals were collected from the plants being maintained in the gardens of the Mandsaur city, India. The rose flowers were

sampled during February to March in 2010. The flowers were picked in morning and stored in at low temperature for further use. Rose flowers of same number patels five petals were randomly selected and drawn the patels. Drawn patels were surface sterilized by dipping in 0.1% mercuric chloride for about 2 min and washing 6 times. By using Soxhlet extraction method, rose patels extract were obtained with various solvents. Here petroleum ether, alcohol and water used as a solvent. All extracted material applied in 1:1, 1:2 and 1:3 dilutions with distilled water

The nutrient agar medium was used for the growth of various strains of pathogenic bacteria. The bacterial cultures were grown overnight in a broth at 37° C using Incubator. The solid medium used was the nutrients plus 1.5% agar (Hi-media). The bacterial lawns were prepared by spread plating 100 m l of overnight grown bacterial culture on the agar plate surface. The test plates were incubated for 24 h at 37° C.

Using a metal borer, 5 mm diameter discs of Whatman filter paper were cut out. The discs were soaked in extract and measure the quantity of extract contained in each disc. The discs were placed on the surface of nutrient agar already plated with test bacteria by using disc diffusion method fro antimicrobial activities. Whatman filter paper discs of the same size, containing 0–15 mg streptomycin per disc were similarly deposited on test plates to serve as controls. The various extract with varying dilutions was applied against various pathogenic bacterial strains. (**Table 1**) Zone of Inhibitions was measured in mm unit.

Bacterial Strains Used	MTCC code
E.coli	1683
Streptococcus pneumoniae	1936
Salmonella typhimurium	98
Enterobacter aerogens	111
Proteus vulgaris	744
Staphylococcus aureus	7443
Staphylococcus epidermidis	3382
Bacillus subtilis	6942
Citrobacter frundii	1658
Pseudomonas Aeruginosa	4673

Result and discussion

As evident from Table 2, the rose petals extract of various solvents showed detectable antimicrobial activities against 10 MTCC bacterial strains (Table 1). At 1:1 dilution of alcoholic extract showed higher inhibition for *Streptococcus pneumoniae* (30 mm), *Enterobacter aerogens* 28 mm, *Staphylococcus epidermidis* 25 mm, *Bacillus subtilis* 30 mm , *Pseudomonas Aeruginosa* 32 mm as compare to *Proteus vulgaris* 22 mm, *Staphylococcus aureus* and *Citrobacter frundii* 25 mm. when study data were analyzed, it is found that alcoholic extract does not showed significant zone of inhibition on *e coli* as compare to other bacterial strains it showed 16 15 and 14 mm zone of inhibition with all dilutions respectively at intermediate dilution of alcoholic extract showed higher inhibition activity against *Streptococcus pneumoniae* 27 mm and *Pseudomonas Aeruginosa* 28 mm. Besides this at higher dilution (1:3) alcoholic extract showed higher inhibition activity against *Enterobacter aerogens*, 26mm *Streptococcus pneumoniae* 27 mm and *Pseudomonas aeruginosa* 29 mm respectively as compare to other bacterial strains. Result data showed that lower dilution of alcoholic extract showed higher antimicrobial activity as compare to higher dilution (**fig 1**).

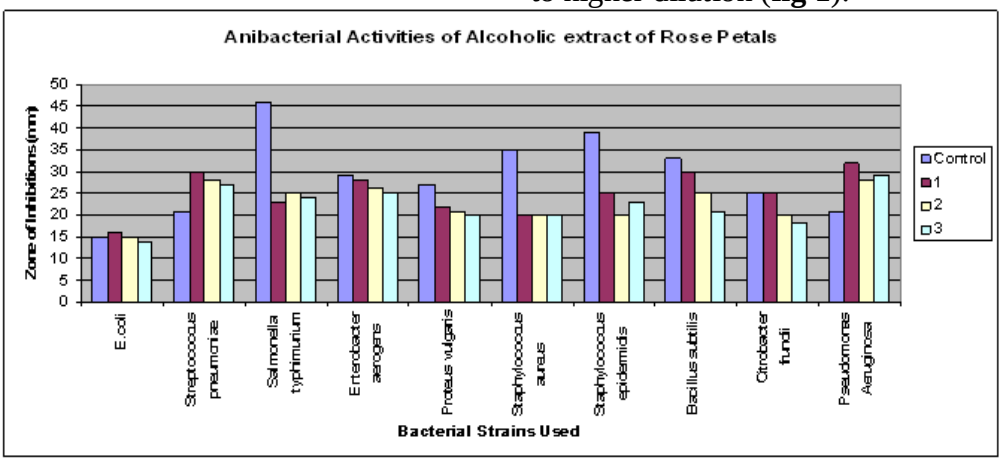

Table 1:- The MTCC bacterial strains used in present study

Similar results also found by Darokar *et a*l in 1998, found the gram-positive *Bacillus subtilis* strain ATCC 6015 and the ATCC 25668 strain of gram-negative bacterium *Pseudomonas aeruginosa* proved to be the most sensitive to rose petals; the petals of 18 varieties were active against ATCC 6015 and those of 13 varieties against ATCC 25668. Among the gram-positive bacteria, *Streptococcus mutans* and *Mycobacterium smegmatis* were relatively more tolerant to rose petals compared to *Staphylococcus epidermidis*, *S. aureus* and *B. subtilis*. Among the gram-negative bacteria, the strains of *Escherichia coli*, *Enterococcus faecalis* and *Enterobacter aerogenes* were more tolerant to rose petals compared to *Klebsiella pneumoniae*, *Salmonella typhimurium* and *P. aeruginosa* strains (Darokar et al in 1998).

In present study when petroleum ether extract of rose used at low dilution (1:1), showed maximum 29 mm zone of inhibition for *Pseudomonas aeruginosa* as compare to other bacterial strains *E.coli* 16 mm, *Streptococcus pneumoniae* 16 mm, *Salmonella typhimurium* 15 mm, *Enterobacter aerogens* 16 mm, *Proteus vulgaris*, *Staphylococcus aureus, Staphylococcus epidermidis*, *Bacillus subtilis and Citrobacter frundii* showed 15 mm zone of inhibition. When data analyzed it found that lower dilution of petroleum ether of rose petals was highly active against *Pseudomonas aeruginosa*. At intermediate dilution of petroleum ether extract showed maximum zone of inhibition for *Bacillus subtilis* (25mm) as compare to other bacterial strains. Antibacterial spectrum of 1:3 dilution showed higher inhibition for that *Proteus vulgaris* 20 mm and *Streptococcus pneumoniae* (21 mm) as compare to *E.coli* 17 mm, *Salmonella typhimurium* 15 mm, *Enterobacter aerogens* 15 mm, *Staphylococcus aureus* 15 mm, *Staphylococcus epidermidis* 16 mm, *Bacillus subtilis* 20 mm *Citrobacter frundii* 15 mm. In present analysis it found that petroleum extract showed more inhibition at lower dilution ranges. When data analyzed it found that lower dilution of petroleum ether of rose petals was highly active against *Pseudomonas aeruginos* (**fig 2**).

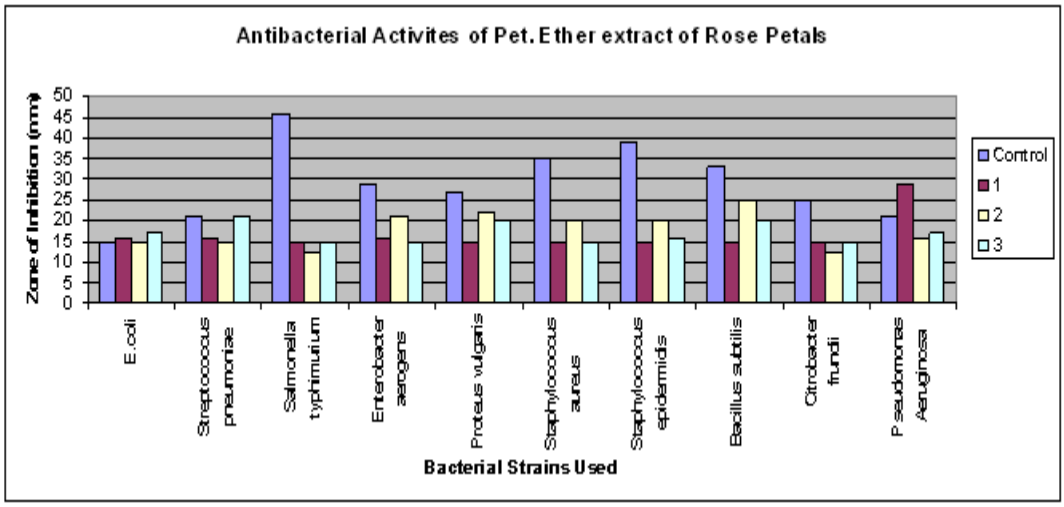

Fig 2- Antibacterial spectrum of petroleum ether rose petals extract (at various dilutions).

Results showed that when aqueous extract used for antimicrobial activity it was found that aqueous extract showed higher inhibition activity against *E.coli* (21 mm), *Enterobacter aerogens (*25 mm) and *Bacillus subtilis* (28 mm) as compare to other bacterial strains. At this 1:1 aqueous extract dilution, inhibitory effect showed against *E.coli* 21mm, Streptococcus pneumoniae 15 mm, *Salmonella typhimurium* 20 mm, *Enterobacter aerogens* 25 mm *Proteus vulgaris* 13 mm, Staphylococcus aureus 13 mm, Staphylococcus epidermidis 15 mm, *Bacillus subtilis* 28 mm, *Citrobacter frundii* 19 mm and *Pseudomonas aeruginosa* showed 15 mm.

Intermediate dilution (1:2) of aqueous extract showed higher inhibition against *Streptococcus pneumoniae* 22 mm, Enterobacter aerogens 20 mm, Staphylococcus *epidermidis* 21 mm as compare to other.At higher dilution (1:3) aqueous extract showed higher inhibition against *E.coli* (25 mm), *Staphylococcus epidermidis* (20 mm) and *Bacillus subtilis* (25 mm). Whereas *Streptococcus pneumoniae*, *Salmonella typhimurium* and *Enterobacter aerogens* showed comparatively less susceptibility against extract.Among all three aqueous dilutions of rose petals extract E.coli, *Streptococcus pneumoniae*, *Enterobacter*

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aerogens, Staphylococcus epidermidis and *Bacillus subtilis* showed high susceptibility(**Fig 3**)

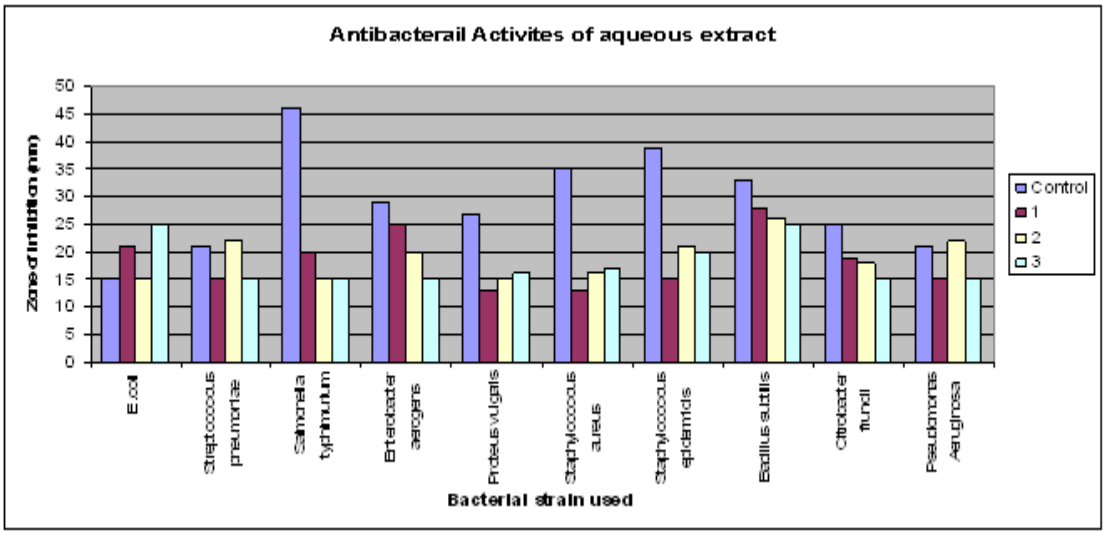

Fig 3- Antibacterial spectrum of aqueous rose petals extract (at various dilutions).

The result analysis showed that alcoholic and aqueous extract showed more potency of antimicrobial activity as compare to petroleum ether extract. The average relative antimicrobial activity was found higher with alcoholic extract (25 mm) as compare to aqueous (19mm) and petroleum ether (18 mm). Statistical data analysis showed that, petroleum ether showed low average relative antimicrobial activity among all there solvents. Results also indicated that at lower dilution extract showed high antimicrobial activity (**Fig 4**).

Study concluded that when comparing the antimicrobial activity of Rose petals with control antibiotic streptomycin it was observed that the zone of inhibition was higher to streptomycin at the highest concentration, in the case of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* when rose petals extracted with alcohol. Whereas as, when extracted with petroleum ether zone of inhibition for *E.coli* and *Pseudomonas aeruginosa* was higher as compare to control. Moreover in aqueous extract the zone of inhibition for *E.coli* was more than control antibiotic solution. As study analysis showed the *E.coli, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* was more susceptible to rose peals. The antibacterial spectrum of rose petals against the different infectious pathogenic bacteria indicated that there must be several different chemicals in the petals of different varieties leading to bacterial inhibition (**Fig 4**).

The finding of antibacterial activity in rose petals in this study indicates its ethno botanical evidences on the use of petals of rose flowers to cure diarrhoeal diseases, opportunistic infection and skin infection.

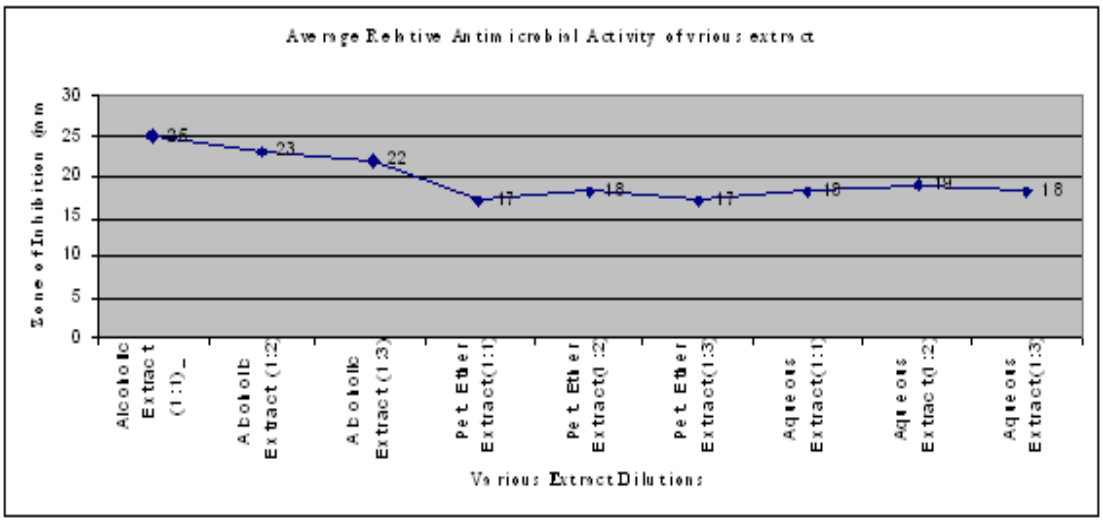

Fig 4 – Average Relative Antimicrobial Activity of Various Extracts.

Table 2:- Antimicrobial Activity of various extracts of Rose patels

Antibacterial activity of various rose patels extract										
	Zone of Inhibition (mm)									
	Control	Alcoholic Extract Dilutions			Pet. Ether Extract			Aqueous Extract		
Bacterial Strain Used		**1:1**	**1:2**	**1:3**	**1:1**	**1:2**	**1:3**	**1:1**	**1:2**	**1:3**
E.coli	15	16	15	14	16	15	17	21	15	25
Streptococcus pneumoniae	21	30	28	27	16	15	21	15	22	15
Salmonella typhimurium	46	23	25	24	15	12	15	20	15	15
Enterobacter aerogens	29	28	26	25	16	21	15	25	20	15
Proteus vulgaris	27	22	21	20	15	22	20	13	15	16
Staphylococcus aureus	35	20	20	20	15	20	15	13	16	17
Staphylococcus epidermidis	39	25	20	23	15	20	16	15	21	20
Bacillus subtilis	33	30	25	21	15	25	20	28	26	25
Citrobacter frundii	25	25	20	18	15	12	15	19	18	15
Pseudomonas aeruginosa	21	32	28	29	29	16	17	15	22	15

CONCLUSION:

Study concluded that when comparing the antimicrobial activity of Rose petals with control antibiotic streptomycin it was observed that, in the case of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa,* alcoholic rose petal extract showed higher zone of inhibition as compare to maximum concentration of streptomycin.

Whereas as, when extracted with petroleum ether, zone of inhibition for *E.coli* and *Pseudomonas aeruginosa* was higher as compare to control (streptomycin). Moreover in aqueous extract the zone of inhibition for *E.coli* was more than control antibiotic solution. As study analysis showed the *E.coli, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* was more susceptible to rose petals. The antibacterial spectrum of rose petals against the different infectious pathogenic bacteria indicated that there must be several different chemicals in the petals of different varieties leading to bacterial inhibition (Fig 4). The finding of antibacterial activity in rose petals in this study indicates its ethno botanical evidences on the use of petals of rose flowers to cure diarrhoeal diseases, opportunistic infection and skin infection.

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