Original Article

Phytochemical Analysis and Anti-Microbial Activities of Rosa Indica Collected from Kohat Pakistan

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ABSTRACT

Objectives: The aim of the research work was to assess out the antimicrobial activity and phytochemicals in medicinal plant, Rosa indica.
Methods: For phytochemical screening different reagents were used while antimicrobial i.e. antibacterial and antifungal activities were checked by employing various bacterial and fungal strains, using nutrient agar at 37◦C and agar tube dilution method, the stock solutions of crude and other three fractions were prepared in DMSO i.e. in 4mg/ 1ml ratio respectively.
Results: All solvent soluble fractions of Rosa Indica showed superlative activity against Streptococcus pneumonia, Shigella flexneri, Proteus mirabilis, Pneumonia, Salmonella typhimurium, Vibia cholera, E. coli, Pneumonia aeruginosa but no activity was recorded against Proteus mirabilis by hexane and chloroform fraction. Similarly the crude extract exhibited complete anti-fungal activity against all fungal strains. Chloroform fraction was active in all fungal strains except Aspergillus fumigatus. The n-hexane fraction was active against Aspergillus fumigatus and Aspergillus Niger while n-butanol fractions exhibited best Antifungal activity against all fungal strains except Aspergillus Niger. The Rosa indica show best phytochemical results.
Conclusion: The above mentioned result recommends that Rosa Indica have excellent anti-fungal as well as anti-bacterial properties and can be used for disease management and therapy. Beside this Rosa Indica have also gain important in scientific ethno-botanical studies because of their phyto-chemicals..

Keywords: *Rosa indica*, Phytochemicals, Antibacterial, Antifungal.

INTRODUCTION

Therapeutic plants are generously available throughout the world. Therapeutic plants are now much more motivated than ever before because they are capable of producing numerous benefits to community indeed to mankind, particularly inside the type of medication. These naturally occurring compounds are believed to form the building blocks of modern-day prescription medication as we recognize today[1-6].

Rose has influenced cultures artistically, economically, clinically, scientifically, psychologically and religiously since manhood could fragrance and appreciates its essences. The presence of anthocyanins in rose petals in high concentration give assurance to these signs because anthocyanins are recognized for their ability of having anti-bacterial, anti-inflammatory and anti-oxidant activity. Beside that it also provides strength to plant's vascular system and reduce blood platelet stickiness[7-12]. All members of *Rosacea* family have been cultivated worldwide. There are approximately 10,000 species of rose, including the most fragrant *R. damascena, R. gallica, Rosa centifolia, R. muscatta, R. indica, R. rubiginosa and R. rugosa*[13]. Amongst all, *R. damascena Mill* is one of the most popular rose specie, it has been commonly employed for the extraction of rose essential oil[14-17].

Literature survey reveals that several studies were published on anti-fungal, anti-bacterial and anti-oxidant activities of roses[18]. The physiological functions of *Rosacea* may be partially due to the abundance of flavonoids and phenolic compounds, also known as bioactive agents [19]. Phenolic compounds retaina wide range of bio-chemical activities, such as free-radical scavengers, anti-bacterial, anti-oxidants[20-22], anti-inflammatory[23], anti-mutagenic[24] and anticancer[25]. Like phenolic compounds, flavonoids also show anti-oxidant activities, they were observed to protect DNA against oxidative damages[26,27]. Beside that they possess strong resistance against UV radiation having wavelength of 254 nm and potentially it is believed that they could be used in anti-solar creams[28].

In a nutshell medicinal plants are the most important source for varieties of drugs because phytochemicals present in these plants are more precise, environment friendly, easily decomposable. Photo-chemical bid exclusive platform for biological functions and structural diversity which are essential for drugs discovery, their biological properties help in reducing the risk of various chronic disease[29-31]. An extensive range of phytochemicals exists within *Rosacea*[32,33].

One of the main problem that concerns open wellbeing, is usually microbial opposition in opposition to antibiotics[34,35]. Consequently, researchers are hectic in screening natural resources for antimicrobial agents yet undiscovered[36-38]. Extract of *Rosa. damascena* were found to have antimicrobial activities[39].This acknowledgement that a lot of contagious pathogenic creatures are building resistance (s) from the relevant medications rapidly offers necessitated to search for new sources of anti-microbial compounds[40]. That's why; the intention of this study was to evaluate the antimicrobial activities and phyto-chemical screening of rose.

MATERIALS AND METHODS

Collection of plant

Rose flowers were collected early morning from Bannu, Khyber Pakhtunkhwa, Pakistan. The sample rose flowers were collected from the plants being kept in the gardens. The rose flowers were collected during February to March in 2014.

Preparation of plant extract with ethanol

After collection rose petals dried under shade for a couple of hours and were then extracted with ethanol. The extract obtained was dried using a Rota-evaporator at 60°C. The extract was further dried at room temperature. After complete drying, it was fractionated consequently with hexane, chloroform, ethyl-acetate and finally with butanol. All solvent fractions were allowed to dry under room temperature.

Bacterial strains and growth conditions

Bacterial strains used in the research were obtained from the microbial collection, from the Department of Microbiology, KUST, Kohat (Table 1). Bacteria were sub-cultured from stock maintained in nutrient agar at 37 ◦C.

Determination of antifungal activity

Using Agar tube dilution method, the stock solutions of crude and other three fractions were prepared in DMSO i.e. in 4mg/1ml ratio. (1 ml from each dilution was added to sterilize each test tube, and then 9 ml of nutrient agar (Sterilized media) was added to the test tubes.). Small piece of previously grown fungus was added to each test tube with the help of sterilizing loop. The tubes containing positive and negative control were incubated at 250 ◦C for a week. After a week the fungal growth in each tube was observed with reference to the positive control[41]. The anti-fungal activities of *R. indica* was checked by using four different fungal strains i.e. A. Flavus, A. Fumi, A. Niger and F. Solani.

*positive control contains fungus, *negative control is without fungus.

Phytochemical analysis

Phytochemical screening of rose extracts were carried out according to the methods described by Arun Joshi, Maya Bhobe and AshmaSattarkar[42], for the detection of bioactive components like alkaloids, carbohydrates flavonoids and saponins etc.

RESULTS ANS DISCUSSION

Most of the journals provide overview about whether plant extract/fractions possess activity against Gram-negative and Gram-positive bacteria and fungi or not. Nevertheless, not all provide details information about the extent of their activity. The consequence of the study reveals that the polar and non-polar solvent fractions of *Rosa Indica* were active against the bacterial strains that are often responsible for infections. Anti-bacterial and anti-fungal activities of *Rosa Indica* are shown intable-1 and table-2. Anti-bacterial activities of *Rosa Indica* were performed against.

Streptococcus pneumoniae, Shigella flexneri, Proteus mirabilis, Pneumonia, Salmonella typhimurium, Vibia cholera, *E. coli*, Pneumonia aeruginosa strains whereas anti-fungal activity was performed against Aspergillus flavus, Fusarium solani, Aspergillus niger and Aspergillus fumigatus strains.

All solvent soluble fractions of *Rosa Indica* showed excellent activity against Streptococcus pneumoniae, Shigella flexneri, Proteus mirabilis, Pneumonia, Salmonella typhimurium, Vibia cholera, *E. coli*, Pneumonia aeruginosa but no result was recorded against Proteus mirabilis by hexane and chloroform fraction. The crude

and butanol fraction showed highest activity against all strains of bacteria. Similarly comprehensive anti-fungal activity was shown by crude against all fungal strains. Chloroform fraction showed zero percent activity against Aspergillus fumigatus. The n-hexane fraction was active against Aspergillus fumigatus and Aspergillus niger while n-butanol fractions exhibited best Antifungal activity against all fungal strains except Aspergillus niger. There are so many reasons to put a question, that why *Rosa Indica* show anti-fungal and anti-bacterial activities. The most effective reason is the presence of various phytochemicals like alkaloids, phenolic acids, flavonoids, tannins, and volatile oils. *Rosa Indica* exhibited best anti-fungal and anti-bacterial activity against such bacterial and fungal strains that are often present in our environment particularly in our food and cause serious infections. It can be used as antimicrobial, for the treatment of different bacterial and fungal diseases and infections.

CONCLUSION

The above mentioned result recommends that *Rosa Indica* have excellent anti-fungal as well as anti-bacterial properties and can be used for disease management and therapy. It may also be used or suggested as a new source in anti-microbial medications and for disease therapy. Beside this *Rosa Indica* have also gain important in scientific ethno-botanical studies because of their phytochemicals. Further research is necessary for isolation and characterization of effective elements of the extracts and also to elucidate the mechanism of their action.

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Table 1. Showing anti-bacterial activity of rose fractions against different strains

S. No	Bacterial Strains used	MTCC code	Crude	Hexane	Chloroform	Butanol	1MP
1	*Streptococcus pneumonia*	6305	14	14	11	10	49
2	*Shigella flexneri*	12022	11	13	12	10	52
3	*Proteus mirabilis*	12453	13	0	0	15	42
4	*Pneumonia*	-	22	11	9	26	29
5	*Salmonella typhimurium*	14028	16	12	9	14	26
6	*Vibia cholera*	9459	24	12	7	28	27
7	*E. coli*	25922	27	11	9	20	19
8	*Pneumonia aeruginosa*	27853	23	10	10	28	26

Table 2. Phytochemical screening

S. No	Phytochemical screening		Inference
1	**Alkaloids**		
a.	Mayer's test	+	Presence
b.	Wagner's test	+++	Presence
c.	Hager's test	++	Presence
2	**Carbohydrates**		
a.	Molish's test	+++	Presence
b.	Benedict's test	++	Presence
c.	Fehling's test	+++	Presence
3	**Flavanoids**		
a.	Lead acetate test	++	Presence
4	**Triterpenoids &Steroids**		
a.	Salkowshi test	++	Presence
5	Phenolic compounds	+++	Presence
6	Resins	+	Presence
7	**Proteins**		
a.	Biuret's test	-	Absence
b.	Millen's test	-	Absence
8	**Glycosides**		
a.	Legal's test	++	Presence
b.	Liebermann's test	+	Presence
9	**Saponins**		
a.	Foam test	-	Absence

Table 3. Anti-fungal activity of rose fractions

Fractions	*A.Fumi*	*A.niger*	*A.Flavos*	*F.Solani*
Curde	**+**	**+**	**+**	**+**
n-Butanol	**+**	**-**	**+**	**+**
n-Hexane	**+**	**+**	**-**	**-**
Chloroform	**-**	**+**	**+**	**+**

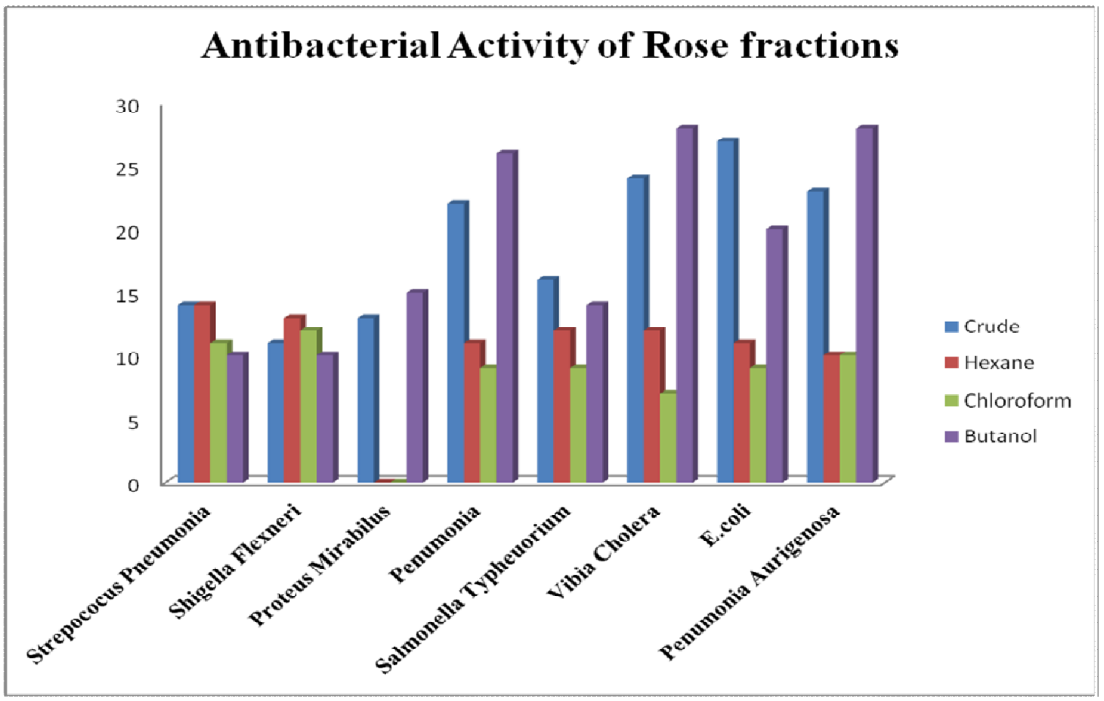

Figure 1. Antibacterial activity of rose fractions