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Full Length Research Paper

Antimicrobial screening of fruit, leaves, root and stem of *Rubus fruticosus*

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Current investigation has been carried out to evaluate antimicrobial potential of an indigenous medicinal herb *Rubus fruticosus* fruit, leaves, root and stem. Crude methanolic extracts were screened against eight bacterial strains for example *Escherichia coli*, *Salmonella typhi*, *Streptococcus aureus*, *Proteus mirabilis*, *Micrococcus luteus*, *Citrobacter*, *Bacillus subtilis* and *Pseudomonas aeruginosa* by Kirby-Bauer method. Leaves showed zone of inhibition at minimum dose level, root extract has comparable results to the standard Ampicillin applied. Minimum inhibitory concentration (MIC) values against bacteria were determined, the stem extract has 20 µg against all strains tested, the rest has different MIC values against different strains. The order of potency on MIC basis is stem> root> leaves> fruit. The antifungal activity were monitored against nine pathogenic fungal strains, *Aspergilus parasiticus*, *Aspergilus niger*, *Yersinia aldovae*, *Candida albicans, Aspergillus effusus*, *Fusarium solani*, *Macrophomina phaseolina*, *Saccharomyces cerevisiae* and *Trichophyton rubrum*, but there is no significant antifungal activity.

Key words: *Rubus fruticosus*, antimicrobial screening, minimum inhibitory concentration.

INTRODUCTION

Rubus fruticosus (Rosaceae) locally known as “Karwara” (Zabihullah et al., 2006), “Akhara” (Ajaib et al., 2010), “Ach” (Sher and Hussain, 2009) and “Baganrra” (Sher et al., 2010) grows wild in Northern areas of Pakistan like Malakand (Zabihullah et al., 2006; Sher, 2011), Kotli (Ajaib et al., 2010), Chitral (Ahmad et al., 2006), Dir (Jan et al., 2008) and Mansehra (Shah and Khan, 2006). It is well known due to its nutritional and medicinal importance by indigenous communities. Fruits are edible and used in jams and jellies (Humayun, 2003). A tea is prepared from the dried leaves (Bown, 1995). Its young shoots are used in salads (Phillips and Foy, 1990). Its leaves are used as fodder for cattle (Humayun, 2003) and plant is used as fences. Leaves are believed to possess diuretic, carminative and antidiabetic properties and used to cure diarrhoea, cough, fever, haemorrhoids and cystitis (Grieve, 1984; Ozarowski and Jaroniewski, 1989; Borkowski et al., 1994; Bown, 1995; Humayun, 2003; Sher, 2011). A decoction of the leaves is useful as a gargle in treating thrush, mouth ulcers and gum inflammations (Chiej, 1984; Bown, 1995; Chevallier, 1996). The leaves are also used to treat skin and gastrointestinal tract infections (Ozarowski and Jaroniewski, 1989; Borkowski et al., 1994) and wounds in animals (Simoni and Guarrera, 1994). Leaves are used for digestive disorder of calves and piglets (Enache and Iancu, 1981) besides being part of herbal deodorant composition (Kazumasa et al., 2003). Decoction of root-bark is used in diarrhoea and dysentery (Jan et al., 2008). The root-bark and the leaves are strongly astringent, depurative, tonic and vulnerary (Launert, 1981; Chiej, 1984; Grieve, 1984; Mills, 1988; Chevallier, 1996). The decoction of root is also useful against whooping cough in its spasmodic stage (Sher, 2011). Its fruit functions as a laxative (khan et al., 2010).

Ripened fruit is eaten to control stomachache and to enhance digestion (Sher et al., 2010). Its ripened fruit in combination with leaves of *Achyranthes aspera* is applied in eye diseases locally called as “Phola” (Shah and Khan, 2006). Despite tagged antimicrobial potential of its various parts, very few published work is available on its antimicrobial activities (Rios et al., 1987; Djipa et al.,

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2000; Cavanagh et al., 2003). As part of continuous studies on exploring hidden potential of indigenous flora of Pakistan (Zia-Ul-Haq et al., 2007a, b, 2008a, b, 2009, 2010, 2011) we have screened methanolic extract of various parts of *R. fruticosus* against some common bacterial and fungal strains.

MATERIALS AND METHODS

R. fruticosus fruit, leaves, root and stem were collected from Dir (L) district, KPK, Pakistan and identified by Prof. Dr. Mansoor Ahmad. A voucher specimen number RIPS-0012 was deposited in herbarium at Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi. The plant parts were chopped and dried in shade to prevent photochemical degradation and to avoid fungus growth. Dried plant parts were soaked in methanol for 15 days. The methanolic extract were filtered and evaporated under vacuum to obtain a thick gummy mass. The extracts thus obtained were used to assess antibacterial and antifungal capacity.

Antibacterial activity

Eight bacterial strains namely: *Escherichia coli*, *Salmonella typhi*, *Streptococcus aureus*, *Proteus mirabilis*, *Micrococcus luteus*, *Citrobacter*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were used for antibacterial assays. Disc diffusion method (Bauer et al., 1966) was used to investigate the susceptibility of methanolic extract of *R. fruticosus* against these strains. Filter paper discs (Whatman; 8 mm diameter) were soaked in extract and then blotted on a sterile paper towel and placed on the inoculated plates. Ampicillin and amoxicillin were used as positive control while distilled water soaked disc were used as negative control. The zones of inhibition were recorded (Table 1) and minimum inhibitory concentrations (MIC) were calculated (Table 2).

Antifungal activity

Antifungal activity was carried out using 9 pathogenic strains of fungi, *Aspergilus parasiticus*, *Aspergilus Niger*, *Yersinia aldovae*, *Candida albicans*, *Aspergillus effusus*, *Fusarium solani*, *Macrophomina phaseolina*, *Saccharomyces cerevisiae* and *Trichophyton rubrum*. Disc diffusion method so called Kirby-Bauer method was used (Bauer et al., 1966) to check the methanolic extracts of *R. fruticosus* for antifungal potential. 6 doses 10 mg, 20, 25, 50, 100 and 200 µg per disc were applied and results were recorded after 24 and 48 h incubation, 'itraconazole' (Mass Pharma Pakistan Privet limited) 'amphoteracin B' Bristol Meyer Squibb Pakistan were used as standard drugs.

RESULTS AND DISCUSSION

Medicinal flora has always been used by indigenous communities to treat various bacterial and fungal infections due to their low cost, timely availability and blind belief of people on tagged antimicrobial powers of these plants. Scientists throughout globe are engaged in validating the claimed pharmacological activities of these plants. Further, broad use of antibiotics to cure microbial infections has made bacteria and fungi resistant to common commercial antibiotics available in market. Therefore, there is dire need to discover hidden natural treasure in form of plants to cure microbial infections. Our results indicate a promising potential of *R. fruticosus* fruit/leaves/stem against commons pathogens. Methanolic extract of *R. fruticosus* leaves showed zone of inhibition at minimum dose level especially against *E. coli*, *B. subtilis*, *S. aureus* and *P. mirabilis*. Stem extract of the plant had most potent effect compared to leaves. *P. mirabilis* was more sensitive to the stem extract. Antibacterial activity of fruit extract was observed against all strains except *S. typhi*, *E. coli* and *P. mirabilis*. Root extract of the plant had given greater zone of inhibition comparable to that of standard drug at micron dose level. MIC for *R. fruticosus* were observed starting from 5 µg/disc until we reached concentration that inhibit the growth of bacteria, we observed that only extract of the stem and standard drug 'amoxicillin' had MIC of 20 and 10 µg/disc respectively against all strains while the rest of extracts and 'ampicillin' drug had different values against different strains (Table 2). The antibacterial action may be due to the presence of tannins (Djipa et al., 2000; Cavanagh et al., 2003).

Akiyama et al. (2001) and Funatogawa et al. (2004) reported tannins for antibacterial activity in plants (Doss et al., 2009). Flavonoids and tannins are among the important constituents of *R. fruticosus* leaves (Gudej and Tomczyk, 2004). *P. mirabilis*, *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *M. luteus* produce urease enzyme which hydrolyses urea to ammonia that results in kidney stones (Toit et al., 1995). Our results suggest that this pathway may be interrupted by this herb by inhibiting growth of aforementioned microbes and thus ammonia bad odour because of bacteria can also be diminished. Most of the gram positive bacteria for example *S. aureus produce, S. pneumoniae* and *S. griseus* produce 'hyaluronidase' enzyme that has tissue damaging effect especially on skin (Hynes and Walton, 2000) and we found in our study the most promising activity against gram positive and *in vitro* inhibitory activity against 'hyaluronidase' is established (Marquina et al., 2002) so due to dual action, various skin infections may be treated. The results of our study revealed outstanding antimicrobial activity against various pathogens responsible for wide variety of infections. Moreover, this study can be used as a tool for bioactivity guided isolation of pure antimicrobial from the plant. We investigated the methanolic extract of *R. fruticosus* for possible antifungal potential but we had no inhibitory observations to be reported. So we can suggest the plant fungicidal activity for future studies using extracts or fractions of non polar solvents.

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Table 1. Zone of inhibition for *Rubus fruticosus.*

Dose µg/disc	Bacterial strains							
	E. coli	***S. typhi***	***S. aureus***	***P .mirabilis***	***M. luteus***	***Citrobacter***	***B. subtilis***	***P. aeruginosa***
20								
F	-	-	8.1±0.16	-	8±0.08	8±0.14	8±0.09	8±0.11
L	8±0.13	-	8.2±0.10	-	-	-	-	-
S	9.2±0.15	8.9±0.11	8.8±0.11	9.8±0.09	8±0.12	9±0.11	8±0.17	9±0.15
R	8.7±0.11	9.2±0.09	8±0.17	8±0.12	8±0.16	-	10±0.11	-
Amp	-	-	-	-	-	-	-	-
Amox	13.8±0.25	12.3±0.12	-	14.6±0.22	14.6±0.19	13.8±0.23	13.2±0.09	8.2±0.15
40								
F	-	-	8±0.17	-	8±0.12	8±0.11	8±0.10	8±0.12
L	10±0.09	-	9±0.12	9±0.09	-	-	7±0.09	-
S	9±0.11	8±0.08	8.5±0.09	10±0.12	9±0.10	8±0.10	9±0.11	9.5±0.13
R	9±0.12	9±0.11	8±0.12	8±0.17	8±0.13	-	10±0.16	9±0.11
Amp	-	-		-	-	-	-	-
Amox	15.8±0.20	12.7±0.16	12±0.09	14±0.1	12±0.09	12±0.19	12±0.11	11±0.09
60								
F	-	-	8±0.13	-	8±0.09	8±0.17	8±0.08	8±0.09
L	11±0.12	-	8±0.10	9±0.19	-	-	7±0.09	-
S	8±0.10	9±0.09	9.5±0.14	8±0.12	8±0.11	8±0.10	8±0.11	9±0.19
R	9±0.09	9±0.12	8±0.10	9±0.12	9±0.16	8±0.09	9±0.19	9±0.2
Amp	-	-	-	10.8±0.09	10±0.14	10±0.13	-	-
Amox	20±0.11	15±0.22	-	16±0.2	15±0.3	11±0.23	14±0.11	12±0.17
80								
F	-	-	8±0.12	-	8±0.16	8±0.12	8±0.10	9±0.2
L	12±0.02	-	8±0.2	10±0.13	-	-	7±0.09	-
S	9±0.09	9±0.12	9±0.17	8±0.2	9±0.19	8±0.09	8±0.11	9±0.20
R	10±0.1	9±0.15	8±0.08	13±0.10	13±0.1	11±0.3	10±0.13	11±0.11
Amp	-	-	-	15.2±0.3	11±0.2	-	10±0.09	-
Amox	18±0.12	14±0.16	-	17±0.29	15±0.19	13±0.26	11±0.22	11±0.13
100								
F	-	-	8±0.2	-	8±0.09	10±0.11	8±0.09	10±0.18
L	11±0.11	-	9±0.21	9±0.13	8±0.07	-	8±0.05	-
S	8±0.2	10±0.09	8.5±0.19	10±0.16	8±0.1	8±0.12	9±0.2	11±0.12
R	10±0.30	8±0.09	8±0.11	13±0.2	13±0.20	9±0.09	9±0.15	10±0.09
Amp	11.2±0.2	-	-	15.3±0.19	14±0.12	-	-	11±0.21
Amox	15±0.15	15±0.23	13±0.33	17±0.2	15±0.23	12±0.28	12±	14±0.20
1000								
F	8±0.08	10±0.2	10±0.22	-	9±0.19	11±0.21	9±0.2	10±0.11
L	11±0.19	8±0.09	8±0.08	10±0.09	9±0.15	11±0.12	9±0.1	10±0.16
S	11±0.11	10±0.11	10±0.21	15±0.17	11±0.2	11±0.15	10±0.19	11±0.24
R	13±0.13	12±0.13	11±0.11	17±0.1	17±0.1	12±0.19	10±0.21	12±0.12
Amp	10.5±o.12	-	-	15.7±0.11	-	10±0.09	11±0.23	12±0.08
Amox	20±0.16	16±0.11	18±0.23	18±0.22	16±0.25	13±0.18	17±0.27	14±0.17

Table 1. Contd.

2000								
F	9±0.09	11±0.2	11±0.23	8±0.09	11±0.18	12±0.1	11±0.09	11±0.26
L	11±0.12	9±0.09	10±0.16	10±0.2	10±0.10	12±0.11	10±0.05	10±0.18
S	11±0.2	11±0.13	11±0.12	15±0.19	11±0.12	12±0.2	11±0.17	11±0.10
R	13±0.08	13±0.09	12±0.13	17±0.12	17±0.17	13±0.23	12±0.2	12.5±0.13
Amp	12.1±0.19	12.1±0.08	-	16±0.09	18±0.15	11±0.19	11±0.09	12±0.21
Amox	20±0.23	20±0.22	20±0.19	20±0.29	17±0.3	13±0.15	14±0.2	12±0.07

F = fruit extract, S = stem extract, L = leaves extract, R = root extract, Amp = ampicillin, Amox = amoxicillin.

Table 2. MIC values for ME extract of *Rubus fruticosus* and standard drugs.

Type of drug applied	Minimum inhibitory concentration in microgram							
	E. coli	***S. typhi***	***S. aureus***	***P. mirabilis***	***M. luteus***	***Citrobacter***	***B. subtilis***	***P. aeruginosa***
F	1000	1000	20	2000	20	20	20	20
L	20	1000	20	40	100	1000	40	1000
S	20	20	20	20	20	20	20	20
R	20	20	20	20	60	20	20	40
Ampicillin	60	100	60	20	40	80	40	40
Amoxicillin	10	10	10	10	10	10	10	10

F = fruit extract, S = stem extract, L = leaves extract, R = root extract.

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