WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 11, 566-573. Research Article ISSN 2277–7105

IN VITRO ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF RUBUS LACIOCARPUS LEAVES

Sandhya Dogra[1], Seema Vidyarthi[1], Subhash Chandra*[2] and Sarla Saklani[2]

[1]Department of Microbiology, Sai Institute of Paramedical & Allied Sciences Dehradun.

[2]Department of Pharmaceutical Chemistry H. N. B. Garhwal (A Central University) Srinagar, Garhwal Uttarakhand India, Pin. Code- 246174.

Article Received on
14 April 2018,
Revised on 04 May 2018,
Accepted on 25 May 2018

DOI: 10.20959/wjpr201811-11766

***Corresponding Author**
Dr. Subhash Chandra
Department of Pharmaceutical Chemistry H. N. B. Garhwal (A Central University) Srinagar, Garhwal Uttarakhand India, Pin Code- 246174.

ABSTRACT

In the present study aimed at evaluating the *in vitro* antimicrobial activity and phytochemical screening of *Rubus laciocarpus* leaves were investigated by standard method. The leaves have been found to potent in antibacterial and antifungal activity (22 mm and 12 mm) respectively. The results showed that the minimum inhibitory concentration (MIC) of *Rubus laciocarpus* leaves extract was 200µg/ml against *Shigella flexneri*. The phytochemical screening of plant for the presence of carbohydrates, glycosides, flavonoids, phenols, saponins, resin and tannins. However, alkaloids were absent. This analysis revealed that, the leaves contained potent value of antibacterial and antifungal activity.

KEYWORDS: Antimicrobial Activity, Kali Hisar and Phytochemical Screening.

INTRODUCTION

The Garhwal region of Uttarakhand is highly enriched with medicinal plants. Such plants are highly potential with their traditional value and medicinal value due to the presence of bio-actives, primary & secondary metabolites. Medicinal plants and herbs are of great importance to the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers**.** One of the great advantages of these medicinal plants is that they are easily available and have moderate side effects.[1&2] *Rubus laciocarpus* belongs to the family of Rosaceae which is commonly known as black Hinsar in Uttarakhand India. Medicinal plants are very important for the well being of rural populations in the region of Garhwal Himalaya, it is not only as sources of food supplemental, nutritionally balanced diets,

medicine, fodder and fuel but also for their income generating potential. *Rubus laciocarpus* fruits have show's astringent, diuretic, anti-diarrheal and anti-dysenteric properties.[3&4] The fruit of *R. laciocarpus* is contains protein, crude fat, vitamin C, minerals and dietary fibers etc. Which is used in the treatment of digestive disorder, astringent, supplementary food, and cardiac disorder and blood disorders.

MATERIALS AND METHODS

Collection and Identification: The materials included fresh and dry leaves of *Rubus laciocarpus* were collected from Rambada district Rudraprayag, Uttarakhand during June-August 2015. These plants were authenticated from Taxonomy Laboratory, Department of Botany, HNB Garhwal (A Central University) Srinagar. The voucher specimens GUH 7842 were deposited in the University herbarium for future records.

***Rubus laciocarpus* leaves**

Chemicals and Experimental instruments: All the chemicals and reagents of analytical grade such as ethyl alcohol (Merck, Bangalore, India) and methanol (Himedia, Chemicals, Mumbai, India) were procured from the respective companies and were used in the study.

Preparation of plant extracts: The leaves were first shade dried for a week. Then the crushed leaves were ground into coarse powder with the help of a mechanical grinder and soxhlet extracted with petroleum ether, chloroform, methanolic and water using the soxhlet apparatus.[5] Each extract was evaporated to dryness under reduce pressure using a rotary evaporator. The extracts thus obtained were stored in air tight container at 4^{o}C until further analysis.

Media: Nutrient broth, Nutrient agar, Muller Hinton agar, Malt extract broth and Sabouraud dextrose agar, Alcohol, Hydrochloric acid, alcohol, and sulphuric acid, Distilled water etc all product of Himedia Laboratories Mumbai (India) were used in this study.

Bacterial Strains: The microorganisms (*Klebsiella pneumonia, salmonella entericatyphim, Staphyloccus aureus, staphyloccus epidermidis* and *streptococcus pyogenes)* used in this investigation were obtained from the culture collection & gene bank, institute of microbial technology, Chandigarh, India, (Customer no. 3921).

Fungal Strains: Three fungal strains were used namely *Candida albicans, Aspergillus flavus* and *Aspergillus parasiticus* used in this investigation were obtained from the culture collection & gene bank, institute of microbial technology, Chandigarh, India, (Customer no. 3921).

Antibacterial assay: The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts.[6&7] Diluted bacterial culture (100µl) was spread over nutrient agar plates with a sterile glass L-rod. 50µg/ml and 100µg/ml of the each sample were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each sample was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37°C for 24 h. After incubation, the diameter of inhibition zones was measured with a caliper.

Antifungal assay: The antifungal activity was tested by disc diffusion method.[8&9] The Sabouraud dextrose agar plates were each similarly seeded with each fungal strain The 24 hrs. both culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45°C respectively, and fungal plates were incubated at 25-28°C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

Phytochemical analysis: The qualitative phytochemical analysis of all samples was carried out using standard methods. The extracts obtained as above are then subjected to qualitative tests for the identification of various plant constituents. In addition, 50 gm of air dried or fresh plant material is also subjected to hydro-distillation to detect the presence of volatile oil. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents on the following lines.[10]

Statistical analysis: The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Tukey's t-test was used as the test of significance. P value<0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software.[11]

RESULTS AND DISCUSSION

In this study, we evaluated the in vitro antibacterial, antifungal and phytochemical analysis of the different extract of the leaves of *Rubus laciocarpus* by standard methods. The result of antibacterial, antifungal and phytochemical analysis of the leaves different extracts of *Rubus laciocarpus* against AOAC methods shown in [Table 01 02 & 03] and [Fig. 01]. The leaves have been found to potent in antibacterial, antifungal and phytochemical analysis of these plant leaves for the presence of carbohydrates, glycosides, terpenoids, flavonoids, phenols, saponins, resin and tannins respectively.

Phytochemical screening: This qualitative chemical test of *Rubus laciocarpus* fruit powder shows the presence of alkaloids, glycosides, carbohydrates, steroids, flavonoids, polyphenols, saponins, resin and tannins.

Table 1: Antibacterial activity of seven bacterial strains against *Rubus laciocarpus* plant leaves extract, Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Bacterial Name		Erythromycin	Petroleum ether Extract		Chloroform Extract		Methanol Extract		Water Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
Klebsiella pneumonia	432	11	-	-	-	10	8	11	9	10
Salmonella entericatypm	98	10	-	-	-	-	9	10	-	12
Shigella flexneri	1457	10	-	12	-	14	12	22	-	16
Staphyloccus aureus	902	11	-	10	-	12	13	18	12	19
Staphyloccus epidermidis	435	10	-	-	-	-	14	20	-	14
Streptococcs pyogenes	1925	12	-	-	-	8	11	19	9	11
Escherichia coli	443	13	-	10	-	11	10	14	-	13

Table 2: Fungal activities of three fungal strains against *Rubus laciocarpus* plant leaves extract, Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Fungal Name		Ketoconazole	Petroleum ether Extract		Chloroform Extract		Methanol Extract		Water Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg /ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
Candida albicans	3017	10	-	-	-	-	-	9	-	10
Aspergillus flavus	2798	8	-	9	-	-	-	10	-	8
Aspergillus parasiticus	2796	9	-	10	-	-	-	8	-	9

Table 3: Phytochemical screening of *Rubus laciocarpus* leaves, (+) – Present, (-) – Absent.

Test	Pt. ether Extract	Chloroform Extract	Methanolic Extract	Water Extract
Carbohydrates/ glycosides (1) Molish test (2) Fehling test (3) Benedict test	 (-) (-) (-)	 (-) (-) (-)	 (+) (+) (+)	 (-) (-) (+)
Alkaloid (1) Mayer's test (2) Dragondroff test	 (-) (-)	 (-) (-)	 (-) (-)	 (-) (-)
Flavonoids (1) Shinoda/pew (2) Ammonia	 (-) (-)	 (-) (-)	 (+) (+)	 (+) (+)
Saponins	(-)	(-)	(+)	(+)
Tannins (1) Pyrogoll & catechol (2) Gallic acid	 (-) (-)	 (-) (-)	 (+) (+)	 (+) (-)
Unsaturated sterol/triterpenes (1) Liebermann & Burchard test (2) Salkowiskis test	 (+) (+)	 (+) (+)	 (+) (+)	 (-) (-)
Resin	(+)	(+)	(+)	(+)
Phenolics compound (1) Ferric chloride (2) Nitric acid	 (-) (-)	 (-) (-)	 (+) (+)	 (+) (+)
Protein and amino acid (1) Xanthoprotien	 (-)	 (-)	 (+)	 (+)

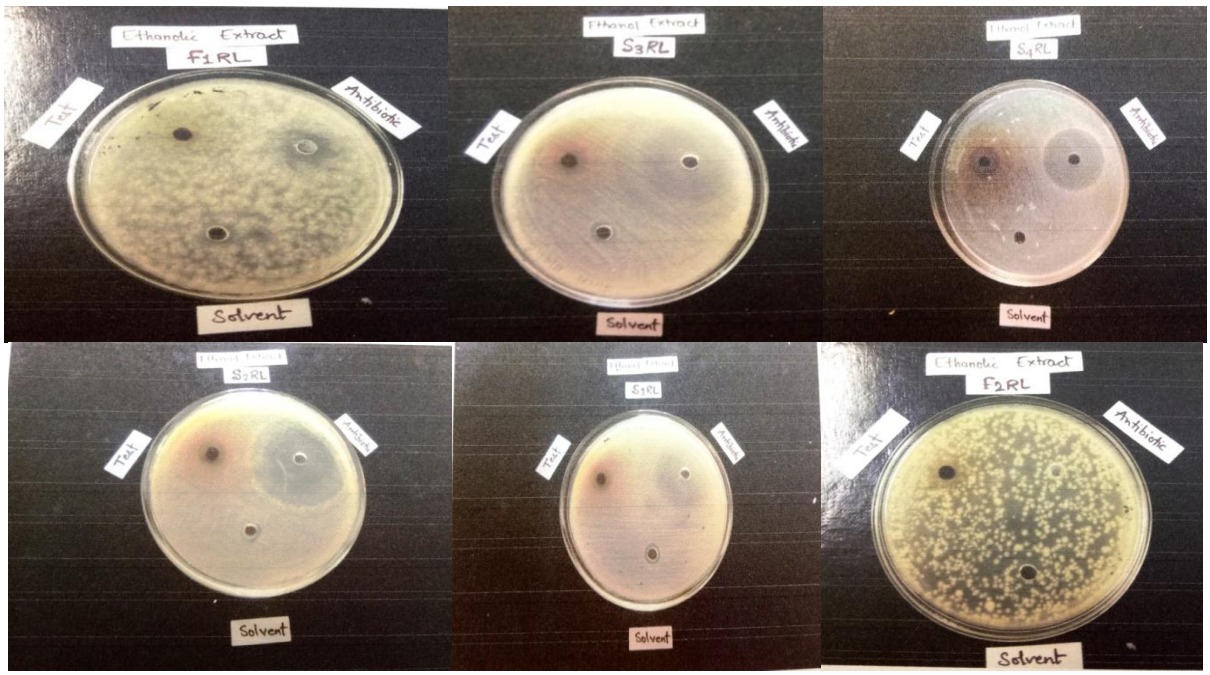

Figure 1 and 2 Antibacterial and antifungal activity of seven bacterial strains & three fungal strains against *Rubus laciocarpus* plant leaves extract.

CONCLUSIONS

It can be concluded that the methanolic fraction of the leaves of *Rubus laciocarpus* possess potent antimicrobial activity and phytochemical screening thus validating the ethno pharmacological claims. This is the first time, to report the above activity present in the methanol extract of *Rubus laciocarpus* leaves. On the basis of our results, *Rubus laciocarpus* appears to have potential for treatment of antimicrobial diseases. It should, however, be explored as a functional medicinal plant for isolating the active ingredients along with animal studies in vivo.

ACKNOWLEDGMENT

The authors are sincerely acknowledged the financial support granted by the UCOST project UCS&T/R&D/CHEM-16/09/10/6539/1, Dehradun and the Deptt. Of Pharmaceutical Science H. N. B. Garhwal (A Central University) Srinagar Garhwal Uttarakhand India.

REFERENCES

1. Subhash Chandra, Sarla Saklani, Evaluation of Garhwal Himalaya Medicinal Plant Rubus laciocarpus Fruit, International Journal of Ethnobiology & Ethnomedicine, 2014; 1(1).
2. Dhani Arya, Major wild edible fruits used by locals of Garhwal Himalaya, Int. J. Adv. Life Science, May 2013; 6: 145-149.
3. Sarla Saklani, Subhash Chandra, Antimicrobial activity, nutritional profile and phytochemical screening of wild edible fruit of Rubus ellipticus, Int. J. Med. Arom. Plants, June 2012; 2(2): 269-274.
4. Meyers K. J, Watkins C. B, Pritts, Antioxidant and anti-proliferative activities of strawberries, Journal of Agri. And food Chemistry, Year 2003; 51: 6887-6892.
5. Lin J, Opak War, and Geheeb-Keller M. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. Journal of Ethnopharmacology, 1999; 68: 267-274.
6. Iennette E.H. Manual of clinical microbiology, 4th edition. American Association for Microbiology, Washington, 1995; 978-987.
7. Rosoanaivo and Ratsimanaga Urverg, Biological evaluation of plants with reference to the Malagasy flora. Monograph for the IFs. NAPRECA Workshop on Bioassays, 1993.
8. Taylor, R.S.L., N.P. Manandhar, J.B. Hudson and G.H.N. Towers, Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethnopharmacol, 1995; 546: 153-159.

9. Espinel Ingroff, A., A. Fothergill, J. Peter, M.G. Rinaldi, and T.J. Walsh, Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for Aspergillus spp.: NCCLS Collaborative Study. Journal of Clinical Microbiology, 2002; 40: 3204-3208.
10. Kokate. C. K., Purohit A. P. and Gokhale S. B, Pharmacognosy, Nirali prakashan 33 edition, 2005; 108-109.
11. Snecdecor GW, Cochran WG, Statistical Mehods, lowa state University press, lowa, USA, 1980; 75.