International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (4): 226-231
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved

Study on Phytochemical Characterization and Antibacterial Activity of Fruit Trees of Chamoli District, Uttarakhand, India

Maneesha Singh[1]*and Rakhi Purohit[2]
[1, 2] **Department of Botany, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun Uttarakhand, India (248001).**

Received: 12 Jul 2019 / Accepted: 14 Aug 2019 / Published online: 1 Oct 2019
***Corresponding Author Email:** singhmaneesha2@gmail.com

Abstract
Some of important local fruit trees of Uttarakhand, India such as *Rubus ellipticus, Berberis aristata, Zizipus mauirtiana* showed astringent and febrifuge properties etc. Ethanobotanically, fruit juices of these plants are sore and used to treat skin diseases, hypertension, cancer, malaria, tranquilizer etc. In continuation of these efforts in search of various biological activities, present study deals with estimation of phytochemical constituents of *Rubus ellipticus, Berberis aristata, Zizipus maurntina* and screened their leaves for their antimicrobial activity. Phytochemical screening of the ethanol extract showed the presence of carbohydrates, tannins and phenolic in all the studied plant samples whereas alkaloid is present in *Berberis aristata and Zizipus maurintina,* and cardiac glycosides was present only in *Rubus ellipticus.* The extract showed potent results against six bacterial strains, *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Bacillus cereus*. Out of these studied plants, *Rubus ellipticus* has highest zone of inhibition and maximum activity against *E. faecalis* and lowest against *E. coli.* MIC analysis was performed by dilution of the concentrated ethanol extract in pure DMSO to achieve a decreasing concentration of 1000µg/ml to 62.5µg/ml. On performing MIC analysis for the ethanol extract of *Rubus ellipticus*, the results showed highest activity against *E. facecalis* (16mg/ml) at 1000µg/ml concentration and minimum of *Salmonella typhi* (10mg/ml). So, it can be concluded that ethanol extract of fruit plants possesses good antibacterial activity that can be enhanced if the phytoactive components are purified and adequate dosage is determined for proper administration.

Keywords
Rubus ellipticus, Berberis aristata, Zizipus maurntina, antibacterial activity, Phytoconstituents

*****

INTRODUCTION:
Biologically active compounds from plant sources have always been of great interest to scientist working on infection disease. Medicinal plants represent a rich source from novel antimicrobial and phytochemical agents. Over the past few decades there has been much interest in plant materials as source of new antibacterial agents. Different extracts from medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against microorganisms, as a result, plants have become one of the bases of modern medicine. The

acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants [1,2]. Other major advantages associated with medicinal plants is better acceptability by the body, because the human body rarely tested them as foreign materials because they are always associated with natural and biological entities like protein, lipid and carbohydrates.

The Indian subcontinent especially Himalayas have a great wealth of medicinal plants and traditional medicinal knowledge. Medicinal plants have played an important role of primary health care system among the local people of Himalayan region. Out of 15,000 species of flowering plants found in India, about 17% have their medicinal value from the Indian Himalayan region [3]. The Central Himalayan region covers the new state (Uttarakhand) of India, provides admirable opportunities for studying the Traditional Knowledge Systems. Uttarakhand has a rich variety of herbs, medicinal and aromatic plant species. Native communities of the area depend on their immediate natural resources for their livelihood besides from primary sectors such as agriculture, horticulture and livestock. Poor development of western system of medicine in this region enforces them to rely on their traditional healthcare practices for maintenance of health as well as diagnosis and improvement of physiological diseases [4,5].

Some of important local fruit trees of Uttarakhand, India such as *Rubus ellipticus, Berberis aristata, Zizipus mauirtiana* showed astringent and febrifuge properties etc [6,7]. A decoction of the root, combined with *Girardinia diversifolia* root and the bark of *Lagerstroemia parviflora*, is used in the treatment of fevers. A paste of the roots is applied externally to wounds. Both the roots and the young shoots are considered to be a good treatment for colic. The leaf buds combined with *Centella asiatica* and *Cynodon dactylon* are pounded to a juice and used in the treatment of peptic ulcers. Most of the studies revealed that extensive investigation was carried out in favour of nomenclature, classification and botanical description and their chemical constituents of these plants. Therefore, the above-mentioned literature survey has prompted us to undertake present study on the phytochemical investigation and antibacterial activity of *Rubus ellipticus, Berberis aristata* and *Zizipus maurintiana* of Chamoli district, Uttarakhand.

MATERIALS AND METHODS

Location of the experiment and climatic conditions

The present investigation was carried at Biotech laboratory in the Department of Botany, School of Basic and Applied Sciences, Shri Guru Ram University, Patel Nagar, Dehradun, Uttarakhand. Located amongst Shivalik Ranges on the foothills of the Himalayas, the Doon Valley is nestled between two of India's mightiest rivers the Ganga on the east and the Yamuna on the west and having the latitude $30^0316'$ N and longitude $78^003219'$ E, covering an area of 300 sq. Km with an elevation of 650 m above the sea level.

Material

The material for the present study comprised of plant leaves of fruit trees of *Rubus elliptieus, Berberis aristata, Zizipus mauritiana* that grows in Chamoli district (29°90' to 31°06' N latitude and 71°10' to 80°15' E Longitude) lies in north-western part of the Uttarakhand state, India.

Experiment methodology

Collection of plant

The fresh leaves of the plants were collected from different places of Chamoli (Uttarakhand). The plant samples were dried in shade at 25°C to 35°C for 15-20 days in the laboratory and then crushed to coarse powder using grinder. The dried plant materials were stored in paper bags.

Extraction

The dried plant material powders were subjected to diffusion extraction with ethanol solvent. About 50gm accurately weighed dry plant sample powder were extracted with 200ml of respective solvent in a closed flask and allowing it to stand for 48 hours. After the extraction process, the filtrate was separated by filtration and the solvent was evaporated using water bath. The extract was then transferred to previously weigh airtight container, (weighed on an electronic balance), percentage yield of the crude extracts was calculated and stored in refrigerator until they were screened for the antibacterial activity.

Phytochemical analysis:

The solvent extracts of *Rubus elliptieus, Berberis aristata, Zizipus mauritiana* were subjected to preliminary qualitative phytochemical investigation for alkaloids, proteins, carbohydrate, cardiac glycosides, tannins, phenolics.

Antibacterial assay:

Antibacterial activity was demonstrated using disc diffusion method [8]. A pure colony of each of the test bacterial strains were subcultured into 5mL of nutrient broth using inoculating loop followed by incubation at 37ºC for 3-4 hours. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) and

subjected to preliminary antibacterial screening with the concentration of 1000µg/ml to know whether they were active against the particular bacteria or not. 0.1ml of inoculums was spread on the solidified Nutrient Agar surface. For screening of active extracts, sterile 6mm diameter whatman No. 41 filter paper discs were dipped in the plant extracts and then placed carefully onto the Muller Hinton agar media against the studied bacterial strains. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate antibacterial activity.

Minimum Inhibitory Concentration (MIC) analysis

The MIC value of extracts were determined only against those bacterial strains which showed high sensitivity during the preliminary antibacterial testing, MIC analysis was performed by serial dilution of the active concentrated extract in pure DMSO to achieve a decreasing concentration range of 1000µg/ml to 31.25µg/ml. By using different concentration of the active extract i.e. the growth around the disc with lowest concentration to which the organism was susceptible would be determined as MIC of the extract against the particular organism.

RESULT AND DISCUSSION

The material for the present study comprised of leaves of some locally grown fruit trees collected from Chamoli district, Uttarakhand such as *Rubus ellipticus, Berberis aristata*, *Zizipus maurintina*. These plants used to treat skin diseases, hypertension, cancer, malaria, tranquilizer, Antiurolithic effect etc [9,10,11,12]. In continuation of these efforts in search of various biological activities, present study deals with estimation of phytochemical constituents of Rubus *ellipticus, Berberis aristata, Zizipus maurntina* and screened their leaves for antimicrobial activity. The findings of the present study were discussed under the following heads:

YIELD AND PHYTOCHEMICAL CHARACTERIZATION OF CRUDE EXTRACTS

In the present study, Plant extracts obtained showed different colour appearances. It varies from light green to dark green. Total percentage yield of crude extract varies from 5.20-7.20.

Table1: Yield and appearance of crude extracts from studied plants:

S.No.	Name of plant	Appearance	Weight of extract (gm)	Percentage (%)
1	*Rubus ellipticus*	Dark green	3.6	7.20
2	*Berberris aristata*	Green	2.6	5.20
3	*Zizipus manritiana*	Dark green	3.5	7.00

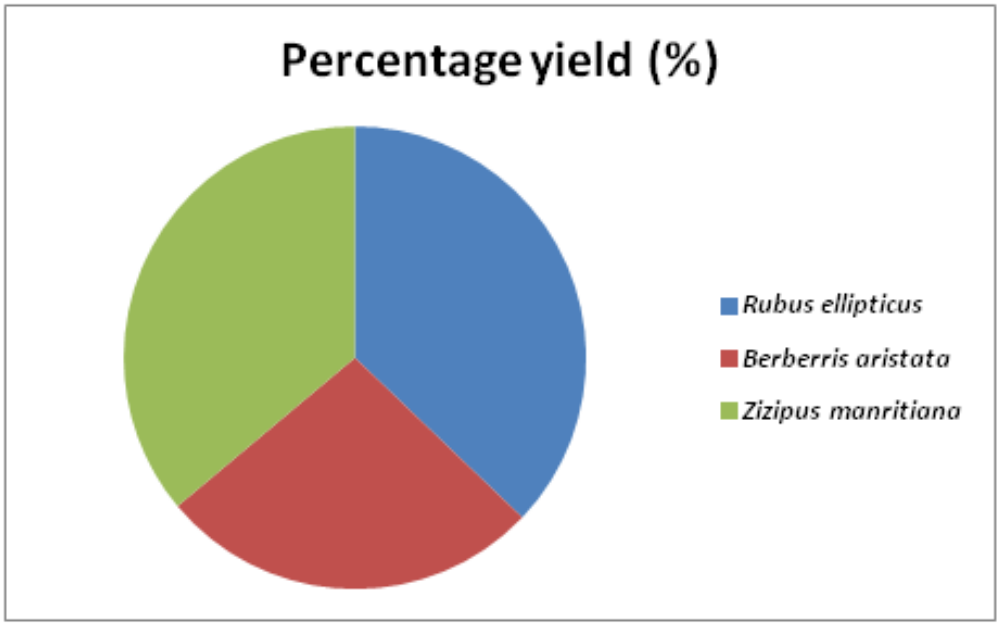

Figure1: Graphical representation of percentage yield of Crude extracts of

Fruit trees extract

Preliminary qualitative phytochemical screening of ethanolic extract of *Rubus ellipticus*, *Berberis aristata, Zizipus maurintina* confirms the presence of carbohydrates, tannins and phenolic compounds in all the plants. Alkaloids was present in *Berberis aristata, Zizipus maurintina* but absent in *Rubus ellipticus*. Cardiac glycosides were present only in *Rubus ellipticus* but absent in other two plant samples.

Table2: Phytochemical analysis of studied plant extract

	Phyto constituents	RE	BA	ZM
1	Alkaloids	-	+	+
2	Tannins and Phenolic	+	+	+
3	Carbohydrates	+	+	+
4	Amino acids	-	-	-
5	Protein	+	-	+
6	Cardiac glycosides	+	-	-

Note: RE: *Rubus ellipticus*, BA: *Berberis aristata,* ZM: *Zizipus maurintina*

ANTIBACTERIAL ASSAY

Plants are important source of potentially useful natural drugs. Many reports are available on the antiviral, antibacterial, antifungal, anthemintic, anti-molluscal, and anti – inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings [13]. The first step to search the hidden natural wealth is in vitro antibacterial activity assay. Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to those plants extracts, which are placed in contact with them.

The extracts were subjected for their preliminary antibacterial screening 1000µg/ml concentration against bacterial strains (three Gram positive and three Gram negative) using disc diffusion methods are used by [8]. The extract showed potent results against six bacterial strains, *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Bacillus cereus.* Out of three studied plant extract, *Rubus ellipticus* plant extract showed the maximum zone of inhibition against the all gram positive and gram-negative bacterial strains except *E. coli* followed by *Zizipus maurintina.* Out of six bacterial strains, maximum antibacterial activity of all the plant extracts were shown against E. *faecalis* bacterial strain followed by *Pseudomonas aeruginosa* minimum activity was shown by *E. coli.*

The ready to use antibiotic impregnated disc Streptomycin (30mcg) were used as a positive control in order to check the sensitivity of the bacterial cultures. This showed clear zones of inhibition around the disc interpreting their high sensitivity towards antibiotics. In contrast to this, DMSO (99% pure) was used as a negative control. Result obtained that was showing sensitivity of the bacteria against positive and negative control is tabulated as follows:

Table 3: Zone of inhibition (mm) of plant extracts against the bacterial strains

S. No.	Bacterial Strains	Zone of inhibition (mm)			Positive control(mm)	Negative control (mm)
		RE	BA	ZM	Streptomycin	DMSO
1	*Bacillus cereus*	13	8	10	40	-
2	*E. coli*	8	8	9	45	-
3	*E. faecalis*	17	12	17	45	-
4	*Pseudomonas aerogenosa*	15	14	13	40	-
5	*Salmonella typhi*	14	9	9	30	-
6	*Staphylococcus aureus*	12	11	12	45	-

Note: RE: *Rubus ellipticus*, BA: *Berberis aristata,* ZM: *Zizipus maurintina*

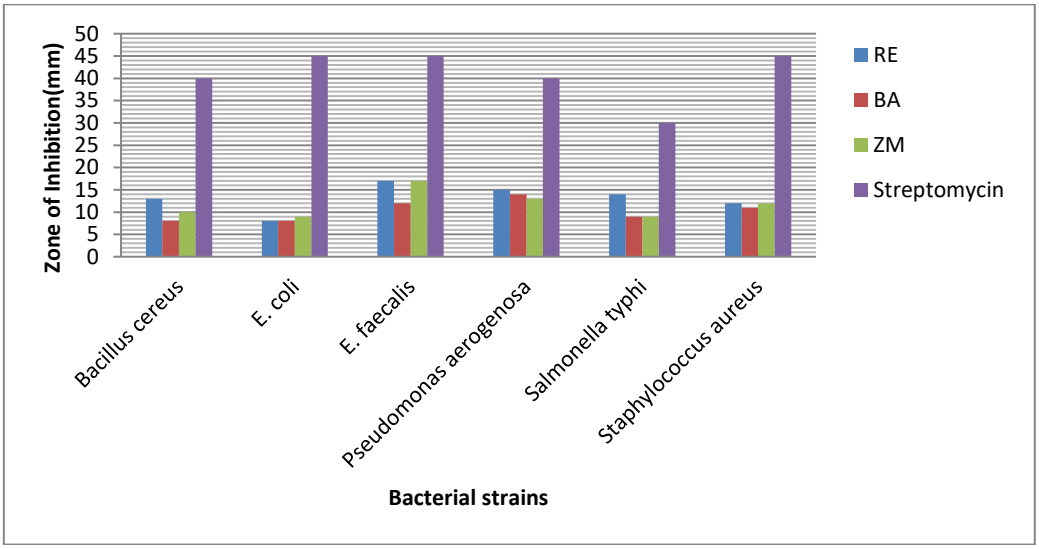

Figure2: Graphical representation of zone of inhibition of crude extracts of Fruit trees extract against bacterial strains

Minimum Inhibitory Concentration (MIC) analysis for ethanol extract

For the ethanol MIC was analysed Minimum inhibitory concentration is important in diagnostic to confirm resistance of microorganisms to an antibacterial agent and also monitor the activity of new antibacterial agents. A lower MIC is an indication of a better antibacterial agent [14].

An MIC are generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism [15]. Out of three studied plant extracts, *Rubus ellipticus* plant extract showed maximum zone of inhibition, therefore MIC was performed of this extract against the bacterial strains. MIC analysis was performed by dilution of the concentrated ethanol extract in pure DMSO to achieve a decreasing concentration of 1000µg/ml to 31.25 µg/ml against *Pseudomonas aerogenosa, Staphlococcus aureus, E. faecalis, S.typhi* . On performing MIC analysis for the ethanol extracts, the results showed that *Pseudomonas aeruginosa* and *E. facecalis* was sensitive against 62.5 µg/ml concentration of crude extract therefore its minimum inhibitory concentration was 62.5µg/ml. Whereas *Staphylococcus aureus* and *S. typhi* were sensitive against 125 µg/ml concentration of crude extract so its minimum inhibitory concentration was 125 µg/ml(table 4). None of the bacterial strain was able to show the zone of inhibition against 31.25 µg/ml which revealed that all of the bacterial strains were resistant against this concentration.

Table 4. Minimum Inhibitory Concentration (MIC) of ethanol extract of *Rubus ellipticus* plant

S.N.	Bacterial Strains	1000µg/ml	500µg/ml	250µg/ml	125µg/ml	62.5 µg/ml	31.25 µg/ml
1	*Pseudomonas aerogenosa*	14	12	10	8	5	-
2	*Staphylococcus aureus*	11	9	8	6	-	-
3	*E. faecalis*	16	14	12	10	8	-
4	*S. typhi*	10	9	8	8	-	-

CONCLUSION

The present study was carried out on the phytochemical characterization and antibacterial activity of the common fruit plants of Chamoli district, Gharwal, Uttarakhand. Phytochemical screening of the extract showed the presence of alkaloids, proteins, carbohydrates, cardiac glycosides, tannins and phenolic. Different extracts of the plant were assayed for antibacterial activity against the pathogenic bacterial strains. Out of these studied plants, *Rubus ellipticus* has highest zone of inhibition and maximum activity against *E. faecalis* and lowest against *E. coli.* On performing MIC analysis for the ethanol extract of *Rubus ellipticus*, the results showed highest activity against *E. facecalis* (16mg/ml) at 1000µg/ml concentration and minimum of *Salmonella typhi* (10mg/ml). So, it can be concluded that ethanol extract of fruit plants possesses good antibacterial activity that can be enhanced if the phytoactive components are purified

and adequate dosage is determined for proper administration.

REFERENCES

1. Chopra I, Hodgson J, Metcalf B, Poste G. 1997. The search for antibacterial agents effective against bacteria resistant to multiple antibiotics Anti microbe Agents. Chemother.4:497-503
2. Jeyachandran R, Mahesh A, Cindrella L, Sudhakar S, Pazhanichamy K. (2009). Antibacterial activity of Plumbagin and Root Extracts of *Plumbago zeylanica* L. Acta Biologica Cracoviensia Series Botanica 51(1): 17–22,
3. Samant SS (1993). Diversity and status of plants in Nanda Devi Biosphere Reserve. In: scientific and ecological expedition to Nanda Devi. Report of Army Head Quarters, New Delhi pp. 54-85.
4. Uniyal SK, Awasthi A, Rawat GS (2002). Traditional and ethnobotanical uses of plants in Bhagirathi Valley, Western Himalaya. Ind. J. Trad. Knowl. 1:7-19.
5. Semwal DP, Saradhi PP, Kala CP and Sajwan BS (2010). Medicinal plants used by local Vaidyas in Ukhimath block, Uttarakhand, Ind J Traditional Knowledge, 9: 480-485.
6. Kala CP (2005a). Indigenous uses, population density and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. Conserv. Biol. 19:368-378.
7. Kala CP (2005b). Local preferences of ethnobotanical species in the Indian Himalaya: Implication for environmental conservation. Curr. Sci. 93:12-25.
8. Dulger B and Ugurlu E. (2005) Evaluation of antimicrobial Activity of Some endemic Scrophulariaceae members from Turkey. Pharmaceutical Biology 43, (3):275-279.
9. Bashir S, Gilani AH. (2011) Antiurolithic effect of berberine is mediated through multiple pathways. Eur J Pharmacol. 651:168-75.
10. Srivastava S, Rawat AKS (2013). Quality evaluation of Ayurvedic crude drug Daruharidra, its allied species and commercial samples from herbal drug markets of India. Evid Based Compl Altern. Med. 472973.
11. Srivastava S, Rawat AKS (2014) Quantification of Berberine in different Berberis species and their commercial samples from herbal drug markets of India through HPTLC. J Adv Chem. 8:1700-6.
12. Srivastava S, Srivastava M, Misra A, Pandey G, Rawat AKS (2015). Review on biological and chemical diversity in berberis (berberidaceae). EXCLI Journal; 14:247-267.
13. Mahesh B and Satish S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences. 4 (S): 839-843.
14. Andrews JM (2001). Determination of Minimum Inhibitory Concentrations. J. of Antimicrobial Chemotherapy. 48(1):5-16.
15. Turnidge JD, Ferraro MJ and Jorgensen JH (2003). Susceptibility test methods: general considerations, In PR Murray, EJ Baron, JH Jorgensen, MA Pfaller, RH Yolken, Manual of Clinical Microbiology. 8th Ed.Washington. America Society of Clinical Microbiology.1103.