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Evaluation of Phytochemical and Antibacterial Activity of Floral Extract of Winter Season Plants in Uttarakhand, India

Research Article

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Abstract

Many plants used today were well known to the people of ancient cultures throughout the world and they were valued for their preservation and medicinal powers. Similarly, Flowers are an essential part of our life, and are wonderful gift of nature, act as a refresher and soothing agents. They are used in Naturopathy and Aromatherapy. The present study deals with the extraction of crude drug from floral samples of four different genera i.e., *Buddhleja madagascariensis Lam., Dendranthema grandiflora, Gladiolus alaska, Callistemon citrinus*(Curtis) Skeel for evaluation of phytochemical and antibacterial activity. Phytochemical screening of these extracts showed the presence of alkaloids, carbohydrates, flavonoids, protein and phenolic compounds while absence of saponin, amino acids in some of samples. Antimicrobial activity studies revealed that *Callistemon citrinus*(Curtis)Skeel plant extract had maximum activity against all the studied bacterial strains. Therefore, Minimum Inhibition Concentration (MIC) of *Callistemon citrinus*(Curtis) Skeel was determined which showed maximum MIC value against *B. subtilis* followed by *S. typii*. On the basis of present studies, it was concluded that plants not having only ornamental values but they can be used for extraction of phytochemicals like alkaloids, flavonoids and phenolic which can be used as antioxidants and antibacterial agents. Thus, in near future these floral extract may be use for the welfare of mankind.

Keywords: Antimicrobial activity; Minimum Inhibitory Concentration; Phytochemicals

Introduction

Plants are accustomed treat a large range of diseases throughout the history of citizenry and this practice continues to this point. Despite the event and growth in conventional medicine, WHO reported that 80% of the Third World population still depend on medicinal plants because the answer to their health problems. This is mainly because most of those herbals are available, affordable and have little or no side effects [1,2]. Phytochemical investigation has proved that indeed plants posses a large range of secondary metabolites, many of which are bioactive against various pathogens, while other compounds have disease preventive properties. Still, others are important in food preservation, as alternative and natural medicine [3]. Drugs derived from plants could also be utilized in their crude form or as semi-synthetic derivatives that manage various ailments. Some naturally medicinal compounds include, ephedrine (bronchodilator), colchicines (antigout), morphine (analgesic) and artemisinin (antimalarial) among others [4]. The anti-microbial properties of plants are investigated by number of researchers worldwide [5,6]. Since antiquity, plants are accustomed

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treat common infectious diseases, the healing potential of the many plants are utilized in Indian traditional medicines like Siddha, Ayurveda and Unani medicine [7]. The rising prevalence of micro-organism showing resistance to antibiotics, there's an urgency to develop new antimicrobial compounds. Infectious diseases represent a very important reason behind morbidity and mortality among the final population, particularly in developing countries. Therefore, pharmaceutical companies are motivated to develop new anti-microbial drugs in recent years, especially because of the constant emergence of microorganisms proof against conventional antimicrobials. Apparently, bacterial species present the genetic ability to amass and transmit resistance against currently available anti bacterial since there are frequent reports on the isolation of bacteria that are known to be sensitive to routinely used drugs and have become multi proof against other medications available on the market [8]. Extracts from the dried flowers and leaves of plants are applied as a paste on wounds in some rural communities. The fresh juice of flowers for e.g. *Catharanthus roseus* was being added within the preparation of tea and also been employed by ayurvedic physicians in India for external use to treat skin problems like eczema and acne [9]. *Chrysanthemum* plants, other than their ornamental value, are highly aromatic because of many volatile components of their essential oils, many of which are utilized in the flavour and fragrance industries. Flower heads are mainly used as chamomile adulterants. While in Japan the leaves are used for suppression of fishy odour in foods like Japanese style soup, yuzu or in Japanese pepper. Buddhleja species are one amongst the important genus of figwort family. It's been used for skin complaints [10] and as abortilacient. A fusion of roots is employed within the treatment of malaria. Gladioli are flowery plants of great economic importance and are mainly cultivated for ornamental and floricultural purposes [11,12]. In some species of Gladioli have various ethnomedical uses like within the treatment of a spread of diseases including hypertension [13]. Gladiolus dalenii is employed to treat gonorrhoea, earache, wounds and disease among others [14]. *Callistemon citrinus* (Curtis) Skeel is very important member of Myrtaceae family commonly referred to as bottlebrush because of the looks of their flowers. *Callistemon* flowers were used as a food source by Australian Arborigines. The flowers were sucked for his or her nectar or accustomed make sweet drink [15].

On the basis of literature and above mentioned survey, it was essential to find out new sources of antimicrobial drugs which can be procured easily without having their side effects and have high economic potential. In this regards, present studies deal with extraction of crude drugs from commonly available floral parts, their preliminary phytochemical potential and antibacterial activity in Uttarakhand, India for treatment of manifestation caused by several pathogenic bacteria.

Materials and Methods

Location of the Experiment and Climatic Conditions

The present investigation was carried out at Biotechnology laboratory, School of Basic and Applied Sciences, Patel Nagar, Dehradun, Uttarakhand located at 30.3165^0 N(latitude), 78.0322^0 E (longitude). Located amongst shivalik ranges on the Foothills of the Himalayas, the Doon Valley is nestled between two of Indias' mightiest rivers the Ganga on the east and Yamuna on the west. The climate of Dehradun is generally temperate, although it varies from tropical, to several cold, depending upon the season, and the altitude of area. The nearby hill regions often get snowfalls during winter but the temperature in Dehradun does not go under 0^0. During summer the temperature here is usually between 27-40^{0}C where as during winter it is between 2-24^{0}C. During monsoon there often constant and heavy rain falls. The main synclinal through receive an average of 210 cm rainfall annually. The agriculture is good here due to the fertile alluvial soil and the adequate water drainage and rainfall.

Material

The material for the present studies comprises of the plant Smoke bush (*Buddhleja madagacarensis* Lam.), Chrysanthemum (*Dendranthema grandiflora*), Gladiola (*Gladiolus alaska*), Bottle brush (*Callistemon citrinus*(Curtis) Skeels).

Experiment Methodology

Collection and extraction of crude plant drug:

The fresh floral parts of present studied plants were collected from different places of Dehradun, Uttarakhand. About 100gm accurately weighed fresh flower plant samples were soaked in ethanol for 72 hours at 26^{0}C. The ethanolic extract were transferred in round bottom flask, leave it for heating at 60^{0}C for 2 hours. The concentrated extract was transferred to the weighed air tight containers and stored in the refrigerator for further screening of phytoconstituents and antimicrobial activity.

Percentage yield

Percentage yield of the crude extract were calculated with the formula:

Percentage yield =weight of extract /weight of powdered drug taken X 100

Phyto-chemical analysis

The crude floral extract of flowers of studied plants was subjected to preliminary qualitative phytochemical investigation. The various test such as alkaloids (Dragendorff's reagent), proteins (Biuret test), carbohydrates (Molisch's test), flavonoids (Lead acetate test), glycosides, amino acids (Ninhydrin test), phenolic compounds (FeCl3 solution), saponins (Foam test) were conducted to determine the presence or absence of the phyto constituents on the basis of colour changes[16].

Sources of bacterial culture

Five pathogenic bacterial strains i.e. two Gram positive-*Staphylococcus aureus* MTCC-737, *Bacillus subtilis*, and three Gram negative bacteria-*Escherichia coli* ATCC-433, *Pseudomonas aeruginosa* ATCC-424 and *Salmonella typhi* MTCC-1255 were procured from Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttrakhand to evaluate antibacterial potential of the floral extracts of different flowers. These bacterial strains were preserved in nutrient agar at 4^{0}C, revived in nutrient broth (liquid medium) and incubated at 37 ^{o}C for 24 hours.

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Anti-bacterial assay procedure:

The anti-bacterial assay performed by the disc diffusion method using pre sterilised disc made from whatman filter paper of diameter 6 mm [17]. The disc diffusion method tests the effectiveness of antibiotics on some specific microorganisms. A pure colony of each of the test bacterial strains were sub cultured into 5mL of nutrient broth using inoculating loop followed by incubation at 37°C for 12 hours. The dried extracts were dissolved in dimethyl sulfoxide (DMSO) and subjected to preliminary antibacterial screening with the concentration of 1000 µg/ml to know whether they were active against the particular bacteria or not. 0.1mL of inoculums was spread on the solidified Muller Hinton agar surface. For screening of active extracts, sterile 6mm diameter what man 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 inhibition concentration (MIC) is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of microorganisms after overnight incubation. In the present experiments, the MIC value of extracts were determined only against those bacterial strains which showed high activity during the preliminary anti-bacterial testing, MIC analysis was performed by serial dilutions of the active concentrated plant extracts in pure DMSO to achieve the decreasing concentration range of 1000µg/ml to 31.25µg/ml. By using different concentration of plant extracts i.e. the zone of inhibition around the discs the lowest concentration of which the bacterial strains were susceptible would be determined as MIC of the plant extracts against the particular bacterial strains. The sensitivities of all the bacterial strains were also observed against the standard drugs such as Streptomycin (15mcg) and against DMSO to set them as a positive and a negative control, respectively.

Result and Discussion

Plants have been used for thousands of years to flavour and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. Currently, data on the antimicrobial activity of numerous plants, so far considered empirical have been scientifically confirmed concomitantly with the increasing number of pathogenic microorganism resistant to antimicrobials. Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment [18]. Flowers are wonderful gift of nature act as a refresher and soothing agents used in naturopathy and aromapathy. Most of anti microbial studies were concentrated on extraction of crude drugs from the vegetative parts of the plants. Studies on floral extract used for antimicrobial activities are very limited. The experimental methodology has been adopted to the present study includes ethanol extraction, concentration of the extracts followed by preliminary phytochemical characterization, anti-bacterial screening against pathogenic bacterial strains and the determination of the MIC value of the active floral extracts against pathogenic bacteria strain. The findings of the present studies were as follows:

Appearance and Yield of Crude Extract:

The appearance of ethanolic crude extract from four different floral samples varied from pale yellow to dark green to red (Table 1). Percentage crude extracts range from 12.21% (*Gladiolus alaska*) to 8.65% (*B. madagascariensis Lam.*). Maximum yield was obtained by *Gladiolus alaska* (12.21%) followed by *Callistemon citrinus*(Curtis) Skeel (10.39%) (Figures 1, 2).

Table 1: Appearance and yield of crude extracts of different floral samples.

S.No.	Floral samples	Appearance of extract	Weight of extract(gm)	%Yield w/w
1.	*Dandrethema grandiflora*	Yellow	9.00	9.00
2.	*B. madagascariensis* Lam.	Dark Green	8.65	8.65
3.	*Gladiolus Alaska*	Pale Yellow	12.21	12.21
4.	*Callistemon citrinus* (Curtis) Skeel	Dark Red	10.39	10.39

Figure 1: Studied Plants for the present study (a) *Gladiolus alaska*, (b) *Buddhleja madagascariensis Lam.*), (c) *Chrysanthemum* (*Dendranthema grandiflora*), (d) Bottle brush (*Callistemon citrinus*(Curtis)Skeels).

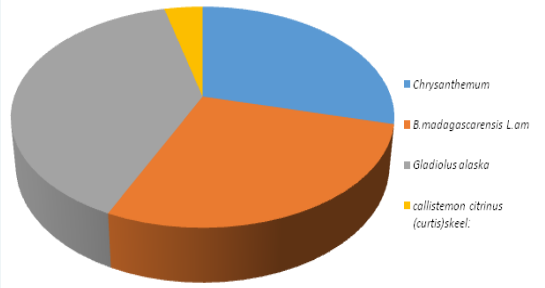

Figure 2: Graphical representation of percentage yield of crude drug extract of floral samples.

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Preliminary Phytochemical Screening

The term phytochemical is generally used to refer to those chemicals which are derived from the plants source. Alkaloids, tannins, flavonoids, saponin, glycoside, terpenoids, steroids etc. are some classes of phytochemicals [19,20]. They play different role in various biological activities like anti-cancer, anti-antioxidant, anti-inflammatory activities etc [21].

On the basis of present studies on preliminary phytochemical characterization of the floral extract revealed the presence of carbohydrates, glycosides, phenolic compounds in all the samples (Table 2). Amino acid and flavonoids were present in *Dendranthema grandiflora* and *Gladiolus alaska* but absent in *Buddhleja madagascariensis* Lam and *Callistemon citrinus*(Curtis) Skeel. Alkaloids and saponins were present in *Gladiolus alaska* and *Callistemon citrinus*(Curtis)Skeel but absent in *Dendranthema grandiflora* and *Buddhleja madagascariensis* Lam. Similarly, the member of Iridaceae family includes flavonoids glycosides, triterpenoids in which flavonoids and their glycosides are found predominantly. Phytochemical screening was also performed on one of *Buddhleja* species i.e. *Buddhleja polystachya* was rich in flavonoids, alkaloids, terpenoides, cardiac glycosides, oils and saponin compounds [22].

Anti-Bacterial Test

Different extracts of floral samples showed anti bacterial activity against all the bacterial strains that are highly pathogenic to human beings causing several diseases such as food poisoning, urinary tract infection, pneumonia, fever etc [14]. Ethanol extracts of floral samples were tested at 1000µg/ml concentration against two gram negative and three gram positive bacterial strains (Table 3, Figure 3).

Table 2: Phytochemical analysis of floral samples

S.No.	TEST	DG	BM	GD	CC
1.	Amino acid	+	-	+	-
2.	Alkaloids	-	-	+	+
3.	Carbohydrate	+	+	+	+
4.	Flavonoids	+	-	+	-
5.	Glycosides	+	+	+	+
6.	Phenolic compounds	+	+	+	+
7.	Proteins	+	+	-	+
8.	Saponins	-	-	+	+

Note: (+), (-) signs indicates the presence and absence of compounds respectively. DG-*Dendranthema grandiflora*, BM-*Buddhleja madagascariensis* Lam., GD-*Gladiolus alaska*, CC-*Callistemon citrinus*(Curtis)Skeel.

Table 3: Zone of inhibition (mm) against pathogenic bacterial strains for floral extracts:

S.N.	Bacterial strains	Zone of inhibition (mm)					
		CS	BM	GD	CC	PC	NC
1.	*Salmonella typhi*	18	07	-	18	22	-
2.	*Bacillus subtilis*	17	16	-	19	30	-
3.	*Escherichia coli*	18	13	-	13	17	-
4.	*Stayphylococcus aureus*	06	06	-	08	20	-
5.	*E . faecelis*	10	-	-	12	20	-

Note: (+), (-) signs indicates the presence and absence of compounds respectively. CS-*Dendranthema grandiflora*, BM-*Buddhleja madagascariensis* Lam., GD-*Gladiolus alaska*, CC-*Callistemon citrinus*(Curtis)Skeel, PC- positive control(*streptomycin*).

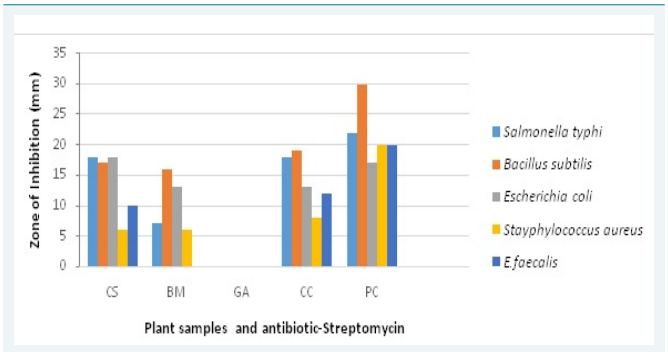

Figure 3: Graphical representation of anti-bacterial screening of all the floral samples.

Antibacterial activity of any sample can be determined by calculating the zone of inhibition. Among the studied floral extract samples, zone of inhibition varied from 6 mm in *Stayphylococcus aureus* to 30 mm in *Bacillus* subtilis, in *Dendranthema grandiflora* zone of inhibition varied from 6mm in *Stayphylococcus aureus* to 18mm in *E. coli* and *S. typhi*. In *Buddhleja madagascariensis* Lam., zone of inhibition ranges from 6 mm in *Stayphylococcus aureus* to 16 mm in *Bacillus* subtilis. Whereas no zone of inhibition was reported against the floral sample of *Gladiolus alaska*. Among the extract assayed, one extract *Callistemon citrinus*(Curtis)Skeel was found to be most active against most of the studied bacterial strains followed by *Dendranthema grandiflora*. The activity against both gram positive and gram negative type of bacteria may be indicative of the presence of broad spectrum antibiotic compounds or, simply metabolic toxins [23].

Similar studies were reported on antibacterial activity of *Callistemon citrinus*(Curtis)Skeel leaves against human pathogens and on *Buddhleja* asiatica against *B. subtilis, S. aureus, P. aeruginosa, E. coli* [24,25]. Anti bacterial activity of Chrysanthemum (leaves, flower, stem, roots) was reported against 14 bacteria and showed some degree of activity against one or more microbial strains [26]. The ready to use antibiotic impregnated disc streptomycin was used as a positive control in order to check the activity of the pathogenic bacterial strains. It showed clear zones of inhibition around the disc interpreting their high activity towards antibiotics. In contrast to this, DMSO (99% pure) was used as a negative control. There was no activity of DMSO against bacterial strains.

Minimum Inhibitory Concentration (MIC) Analysis

Minimum Inhibitory concentration (MIC) is important in diagnostic laboratories to confirms resistance of microorganism to an antimicrobial agent and also to monitor the activity of new anti microbial agents, A lower MIC is an indication of better antimicrobial agent [27, 28].

MIC was performed by serial dilution of the concentrated crude extract of *Callistemon citrinus*(Curtis)Skeel in pure DMSO to achieve a decreasing concentration range of 1000 µg/ml to 62.5 µg/ml (Table 4) (Figure 4). MIC activity reported with 62.5µg/ml for *B. megaterium* and *E. coli* that showed potential efficacy against the bacteria for *Callistemon citrinus*(Curtis)Skeel. Out of five studied bacterial strains, maximum MIC value was reported by *B. subtilis* followed by *S. typii*. Minimum MIC value was reported by *S. auerus*.

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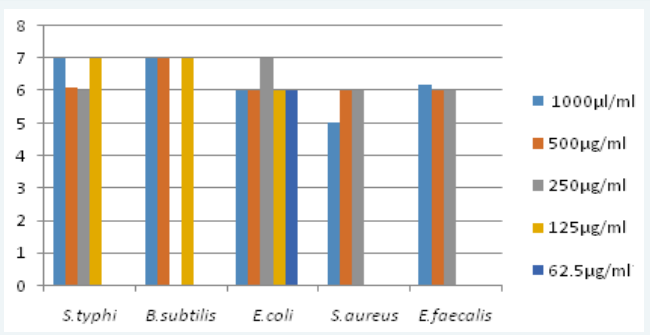

Figure 4: Graphical Graphical presentation of Minimum Inhibition Concentration (MIC) of *Callistemon citrinus*(Curtis)Skeel.

Table 4: Minimum inhibition concentration (MIC) analysis of *Callistemon citrinus* (Curtis)Skeel.

S.N.	Bacterial strain	1000µg/ml	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml
1	*S. typhi*	18	10 mm	07 mm	06 mm	-
2	*B. subtilis*	19	12 mm	09	07 mm	-
3	*E. coli*	13	09 mm	07 mm	06 mm	06 mm
4	*S. aureus*	08	06 mm	06 mm	-	-
5	*E. faecalis*	12	09 mm	07 mm	-	-

Conclusion

On the basis of present study on evaluation of phytochemical screening of the crude extracts showed the presence of secondary metabolites such as alkaloids, flavonoids, carbohydrates, proteins and phenolic compounds in all the studied floral extract and MIC of *Callistemon citrinus* possess good antibacterial activity. This study suggested that plants not having only ornamental values but they can be used for extraction of phytochemicals like alkaloids, flavonoids and phenol which can be used as antioxidants and antibacterial agents. Thus, in near future the floral extract of these plants may be use for various biological activities for the welfare of mankind.

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