RNI No. DEL/1998/4626 ISSN 0973-8507

Vol. I No. 36 JUNE 2024

Universities' Journal of Phytochemistry and Ayurvedic Heights

Herbal Non- antibiotic to combat AMR

Ocimum sp.
(Tulsi)

Emblica officinalis
(Amla)

Alpinia galanga
(Kulanjan)

Hibiscus rosa sinensis
(Gurhal)

Indexed by International Impact Factor Services, Scientific Journal Impact Factor,
International Institute of Organized Research (I2OR), Advanced Sciences Index (ASI),
International Scientific Indexing and Cite factor
Crossref Member
Digital Object Identifier (DOI) Assigned
Website: www.ujpah.in

THE INDIAN CAMBRIDGE SCHOOL

(Affiliated to the Council for Indian School Certificate Examination, New Delhi)

ADMISSIONS OPEN

For Class P.G. to IX

Session Start From: 2019 - 2020

HOSTEL FACILITY FOR GIRLS ONLY

HOSTEL FACILITIES

- EXCLUSIVE NIGHT STUDY AREA.
- MONITORED TV VIEWING OF DAILY ENGLISH NEWS
- AIR CONDITIONED DORMITORIES.
- IN DORM COOL FILTERED DRINKING WATER FACILITY
- 5 TIMES MEALS
- INTERNET /ISD/STD CALL FACILITY.
- SUPERVISED INTERNET ACCESS
- IN CAMPUS TUCK SHOP
- IN HOUSE LAUNDRY

SALIENT FEATURE

- Teacher/ Student Ratio 1:30
- Excellent Science Labs
- Latest Computer Lab with Wi-Fi Facility
- Smart Classes
- Remedial Classes
- Education Tour
- Library With Audio- Visual Rooms
- 24Hrs. Security
- Complete Campus on IT Serveillance

ADMISSION HELPLINE: +91 7249909095/96

12 Chander Road, Dalanwala Dehradun Uttarakhand Pin -248001

Email: info@theindiancambridge.edu.in, Web: www.theindiancambridge.edu.in

Universities' Journal of Phytochemistry and Ayurvedic Heights

Publisher : Phytochemistry and Ayurveda Society (PAS)
1- Inder Road, Dalanwala, Dehradun- 248001, Uttarakhand

Chief Editor / Editor : Dr. S. Farooq / Dr. I. P. Saxena
1- Inder Road, Dalanwala, Dehradun- 248001, Uttarakhand

Email : dr.sfarooq.him@gmail.com / editor@ujpah.in

Website : www.ujpah.in

Telephone : 0135-2640233, 0135-2982845

Disclaimer : Chemical Analysis of herb as described in the journal are based on experimental procedures, various traditional sources & existing available knowledge as established by scholars of Phytochemistry and Ayurveda. Every possible caution with regard to uses affects of these herbs are taken. This journal aims to widely promote knowledge on herbs. Publisher is not responsible for harm caused, if any, by following any use given in the journal.

First Edition : March 2002

ISSN No : 0973-3507

Crossref Member

Contact regarding any enquiry related to publication of research papers, articles etc.

Dr. I.P. Pandey, Co-ordinator
Prof. Emeritus
Universities' Journal of Phyto-Chemistry and Ayurvedic Heights,
1-Inder Road, Dehradun-248001 (Uttarakhand), India
E-mail: dr.sfarooq.him@gmail.com, editor@ujpah.in, ippande@gmail.com, Website;www.ujpah.in

Subscription

Inland annual Subscription	Rs 300/-
Inland annual Scholar's Subscription	Rs 125/-
Inland annual institutional Subscription	Rs 400/-
Overseas annual institutional subscription	$40.00

Advertisement

Full Page	Rs. 10000.00	2 issues
Half Page	Rs. 5000.00	2 issues
Quarter Page	Rs. 2500.00	2 issues

Subscription may be sent by Bank Draft in favour of Universities 'Journal of Phytochemistry and Ayurvedic Heights, 1-Inder Road Dehradun-248001(Uttarakhand), India. The Journal will be dispatched under certificate of posting. An additional amount of Rs. 100. 00 is sent, if delivery is required through registered post.

Editorial

India is rightfully known as the world Pharmacy and ranked third in the production of pharmaceutical products and is currently exporting 40% to USA and 25% to UK of their needs.

The world has started recognizing the power of herbs and its potential through scientific Researches being done. A news article published in the Times of India confirms that scientists are searching once again power of herbs for AMR.

On the other side AMR is a big challenge for our Healthcare Industry. No new antibiotics developed since 30 years. Due to AMR global death rate is 7,00000 death in a year which is alarming and compel us to go for some alternative antimicrobial drugs.

A pesticide known as ethylene oxide. Here's all you need to know about this shocking report. Singapore, Hong Kong and Nepal has banned the import, consumption and sale of spices.

That's not all. The US Food and Drug Administration (FDA) is also investigating products from India containing banned pesticide and putting the human life in danger. Apart now the new variants of Corona Virus commonly compared with pandemic of 2019-20 and 2020-22 is significant but it has been control to a great extent now.

This is the time to review the future of humanity either free from diseases or full of dangerous diseases like Cardiovascular ailments especially "Silent Heart attacks". It has been observed that out of every five heart attacks one is silent heart attack, diabetes, cancers etc.

This issue of the UJPAH focuses the AMR and Herbal Non-antibiotics which needs more research to develop new class of antimicrobial drugs from Herbs.

Research and innovation is the only solution which is required so that better products are to be designed. Dr. Durgesh Pant Director General UCOST has initiated Ayurvedic drug Development within the ambit of Reverse Pharmacology as per guidance laid down by WHO for the development of natural herbal drugs. Uttarakhand is one of the states whose contribution in this field is substantial.

I am personally grateful to Dr. Durgesh Pant Director General UCOST who believes in action.

I am grateful to the UJPAH board members to make this issue a memorable for science fraternity of the Uttarakhand and to all those scientists, research scholars, students and teachers who contributed for bringing out this issue and the people of science at large. My good wishes to all of you who are participating as an author or as a participant for this great event.

Dr. S. Farooq
Chief Editor

Contents

Phytochemical screening and chromatographic studies on Triphala and Trikatu

[1]Sumanlata Chimmwal, [*2]Sakshi Saini and [2]Shailja Tyagi
[1]The Himalaya Wellness Company, Dehradun, UK, India
[*2]DAV Degree College, Muzaffarnagar, UP, India.
[*]**Email: sakshisaini856@gmail.com**

DOI 10.51129/ujpah-2024-36-1(1)

Received – 27 April, 2024
Revised – 1 May, 2024
Accepted – 3 May, 2024
Published –29 June, 2024

Abstract-Triphala and Trikatu both are widely used traditional herbal formulation used in ayurveda for improving digestive disorders, respiratory disorders and in skin disease. Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. Therefore, the objective of the present study was to characterize the phytochemicals profile for various secondary metabolites using HPTLC of Triphala and Trikatu extract. HPTLC studies to explore medicinally active phytoconstituents present in different solvent extracts in these polyhedral formulations.

Key words: Triphala, Trikatu, HPTLC, Photochemical.

Introduction

Ayurveda, the traditional Indian medicinal system remains the most ancient yet living traditions with sound philosophical and experimental basis. According to charaka it is the knowledge which seeks to weigh life in the scales of wholesomeness and happiness against their opposites[(1)]. Ayurveda literally means "The knowledge" of life. In sanskrit the word ayurveda consists of two words 'ayu' meaning 'life' and 'veda' meaning knowledge or 'science'. According to WHO, about 70-80% of the world population rely on non-conventional medicines mainly of herbal sources in their healthcare[(2)].

In this study, we compared and contrasted two different triherbal traditional formulation i.e. triphala and trikatu. Talking about trikatu, trikatu is a sanskrit name indicates its meaning 'tri' stands for 'three' ans 'katu' stands for 'acrids'. Trikatu is the combination of three herbs black pepper (Piper longum), pippali (Piper longum), dry ginger (Zingiber officinalis). Trikatu acts mainly on stomach, liver and pancreas. In stomach trikatu increases the production of digestive juices therefore stimulating digestion. In liver, it is used to increase production of bile salts by stimulating gall bladder. Trikatu affects overall digestive system along with its curative affects on respiratory, urinary immunity, skin and metabolic system of our body[(3)].On the other hand triphala in sanskrit 'tri' stands for 'three' and 'phala' stands for 'fruits' Triphala is the simple equiproportional mixture of three different herbs Amla (Emblica officinalis), Harad (Terminalia chebula), Bahera (Terminalia bellerica).It is classified as a tridoshic rasayana in ayurvedic medicine as it

promotes longitively and rejuvenating in patient of all constituents and ages. Triphala represents an essential foundational formula as it promotes efficient digestion, absorption, elimination and rejuvenation. Moreover, studies have validated a number of potential uses of triphala, which include antioxidant, anti-inflammatory, antibacterial, dental caries prevention, antipyretic, analgesic, anti-mutagenic, anti-cariogenic, anti-stress, hypoglycaemic, anticancer, chemo-protective, chemopreventive, radio protective effects[(4)].

Material and methods

Collection and identification

The plant materials for making of triphala and trikatu were collected from the Himalaya Wellness company campus, Dehradun, UK, India and were identify by the Department of Pharmacognosy. The Himalaya Wellness Company, Dehradun, UK.

Preparation of powder

The collected plant material were dried and powdered using mixer grinder for triphala powder Amla, Harad, Bahera powder were mixed in equiproportional ratio and for trikatu powder Black pepper, Pippali, Saunth powder were mixed in same ratio. In plants alkaloids redox reactions of nicotinamide adenine dinucleotide (NAD) reduces its pyridine ring into dihydropyridine. (Lin SX and Sperry 2020; Pollak and Ziegler 2007).*The plant Senna* spp. is a source of natural alkaloids of the piperidine and pyridine classes.(Francisco et al. 2012)

Extraction of plant material

For phytochemical screening 5g of both sample triphala trikatu dissolve in each solvent water, methanol, acetone, hexane and mixed properly. Leave them overnight and then filtered using whatman's filter paper.

Phytochemical studies

The phytochemical screening of triphala and trikatu in all four solvent (Aqueous, Methanol, Acetone, Hexane) were carried out according to different phytochemicals were estimated qualitatively and quantitatively by using following procedure[(5-8)].

Tests for qualitative analysis

Test for carbohydrates (Molish's test): To two ml of molish's reagent, 2ml of extracts were added and shaken well. To this another 2ml of concentrated sulphuric acid was added carefully through the sides of the test tube. Appearance of a reddish violet ring at the junction of the two layers indicate the presence of carbohydrates.

Test for tannins: To the extracts, a few drops of 10% ferric chloride solution were added. appearance of a green or blue colour indicates the presence of tannins.

Test for steroids: Leaf extracts were mixed with 1 ml of chloroform and 2-3 drops of conc. H_2SO_4were added to it. Appearance of a pink or red colour indicate the presence of steroids.

Test for terpenoids (Salkowiski test): Five ml of the extinct were mixed with 2ml of chloroform and 3ml of conc. H_2SO_4, solution. A reddish-brown colour at the interphase indicate the presence of terpenoids.

Test for alkaloids (Mayer's test): Extracts were treated with mayer's reagent (potassium mercuric chloride). Formation

of a yellow coloured precipitate indicates the presence of alkaloids.

Test for flavonoids: The extracts were treated with conc. H_2SO_4 and formation of a yellowish orange colour indicate the presence of flavonoids.

Test for proteins (Xanthoprotein test): To the leaf extracts 20% NaOH solution were added and the formation of an orange colour confirms the presence of proteins which is characteristic for ammonia formation.

Test for cardiac glycosides (Keller-killani test): Five ml of test extracts were treated with 2ml of glacial acetic acid containing 2-3 drops of ferric chloride solution and 1 ml of conc. H_2SO_4 solution. Appearance of a green ring initially which first turns violet and then to brown at the interphase indicates the presence of cardiac glycosides.

Test for Saponins (Foam test): Two mi of the extracts were diluted with 20ml of distilled water, shaken vigorously and was observed for a stable persistent froth.

Test for phenolic compounds (Ferric chloride test): Two ml of diluted extracts were treated with dil. FeCl, solution. Appearance of a violet colour indicate the presence of phenol like compounds.

Test for triterpenes: The extract were treated with chloroform then add cons H_2SO_4 and shake lower layer changes into yellow colour indicates the presence of triterpenes.

Test for starch: Take 0.015g of iodine and 0.75g of potassium iodide and mix them then add 5ml distilled water and 2ml of extract appearance of blue colour indicates the presence of starch[3].

Test for anthraquinone glycoside: Take extract and add 1ml of H_2SO, and boiled it for 5 min. then filtered it (while hot) and add 1 ml of chloroform, 0.5 ml dilute ammonia. Rose pink to red colour indicates the presence of anthraquinone glycosides.

Chromatographic studies

HPTLC (High Performance Thin Layer Chromatography)

Sample preparation

Aqueous- Measure accurately 3gm of sample in 250ml round bottom flask add 20ml of distill water and reflux it by immersing in a water bath at 80-100^{O}C for 30 minutes. Filter the extracts through whatman no. 1 filter paper into a conical flask.

Methanol-Measure 3gm of sample in 250ml round bottom flask add 20ml of methanol and

reflux it by immersing in a water bath at 70-80^{O}C for 30 minutes. Filter the extract through whatman no.1 filter paper into a conical flask.

Hexane- Measure 3gm of sample in 250 ml round bottom flak add 20ml of hexane and reflux it by immersing in a water bath at 40-50^{O}C for 30 minutes, Filter the extract through whatman no.1 filter paper into a conical flask.

Chromatogram layer- TLC plates, silica gel 60 F254, 10 X 10cm.

Chemicals required-Methanol, Hexane, Chloroform.

Mobile phase- Chloroform: Methanol (90: 10)

Application

Apply the sample and standard solution as 12mm band, in a distance of 12mm from the bottom of a precoated thin layer silica plate of uniform thickness, made a mark up to distance of 8.5 cm from the application point as a development mark using pencil.

Preparation of development tank

Camag made twin through development tank (10X10) was used. Covered one side of the inside chamber with required size of whatman no.41 filter paper. Measured 20ml of mobile phase and transferred into chamber from the side of filter paper.

Visualization and documentation

Visualized the dried plate under UV 254 nm and 366nm using cabinet and capture the image.

Table-1 Detection of secondary metabolites in Polyherbal Trikatu

Class of compounds	Aqueous	Methanol	Acetone	Hexane
Taninn	+	+	+	-
Flavinoid	+	+	-	+
Saponin	+	-	+	-
Alkaloids	-	+	+	-
Starch	-	-	-	-
Protein	+	+	+	+
Carbohydrate	+	+	+	+
Triterpenes	+	-	-	-
Cardiac glycosides	+	+	+	+
Phenolic compounds	+	-	-	-
Steroid	+	-	-	-
Anthra quinone glycosides	-	-	-	-
Terpenoids	+	-	+	-

(+)- Positive: (-)- not Detected

Table-2 Detection of secondary metabolites in polyherbal Triphala

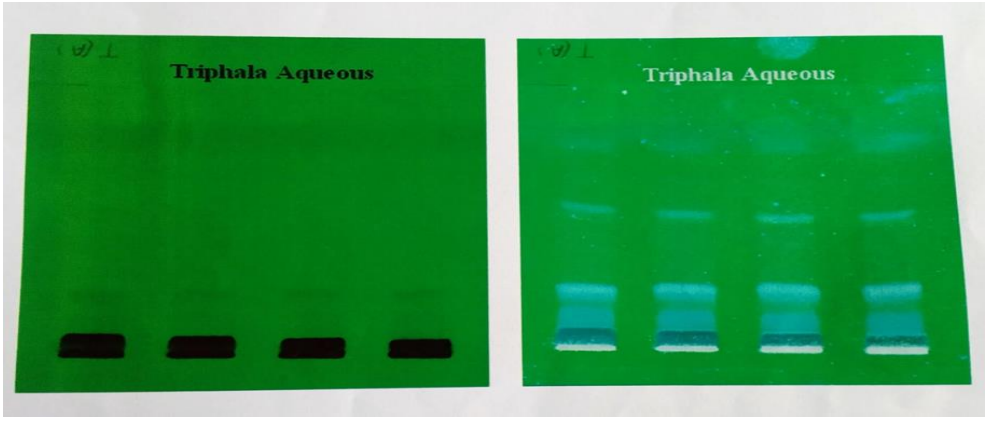
Class of compounds	Aqueous	Methanol	Acetone	Hexane
Taninn	+	+	+	-
Flavinoid	+	+	+	-
Saponin	+	+	+	-
Alkaloids	-	+	+	-
Starch	-	-	-	-
Protein	+	+	+	+
Carbohydrate	+	+	+	+
Triterpenes	+	-	-	+
Cardiac glycosides	+	+	+	+
Phenolic compounds	+	+	+	+
Steroid	-	-	-	+
Anthra quinone glycosides	-	-	-	+
Terpenoids	+	+	+	-

(+)-Positive; (-)-Not Detected

Knowledge of the chemical constituent of plants is desirable because such information may be of great value in revealing new sources of compounds and precursors for the synthesis of new chemical constituent, which can be used in drugs (Mentha et al. 2017). Preliminary analysis is an indication of the presence or absence of phytochemicals in a plant extract based on visual inspection of colour or precipitation reaction; whereas, HPTLC chemo profile accurately and efficiently confirms the presence of these constituents. While traditional TLC is based on visual inspection of the chromatographic plate and its documentation by either tracing or photography, HPTLC features highly sensitive scanning densitometry for rapid chromatogram evaluation and documentation (Mukherjee 2008).

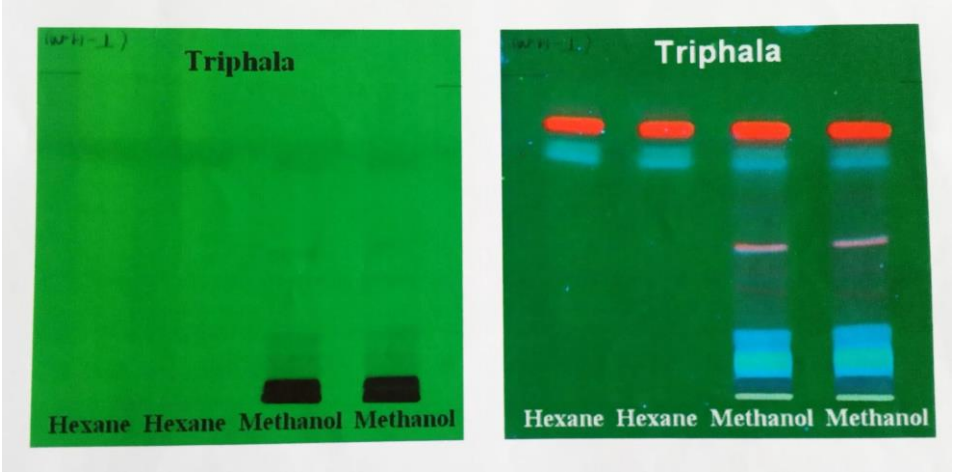
In the current study, Triphala and Trikatu extracts were evaluated for the detection of thirteen main classes of secondary phytocompounds namely Tannin, alkaloids, starch, protein, carbohydrate, flavonoids, saponins, triterpenes, cardiac glycosides, phenolic compound, Anthraquinones glycosides, steroid and terpenoids (**Table-1** and **Table-2)**.

HPTLC of Triphala and Trikatu extract



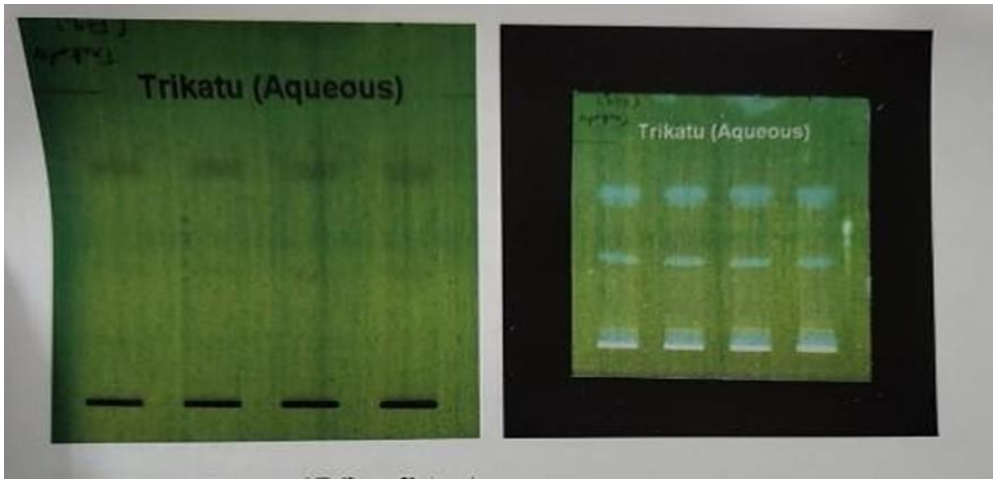
@254nm **@366nm**

Figure: 1 Aqueous extract of Triphala



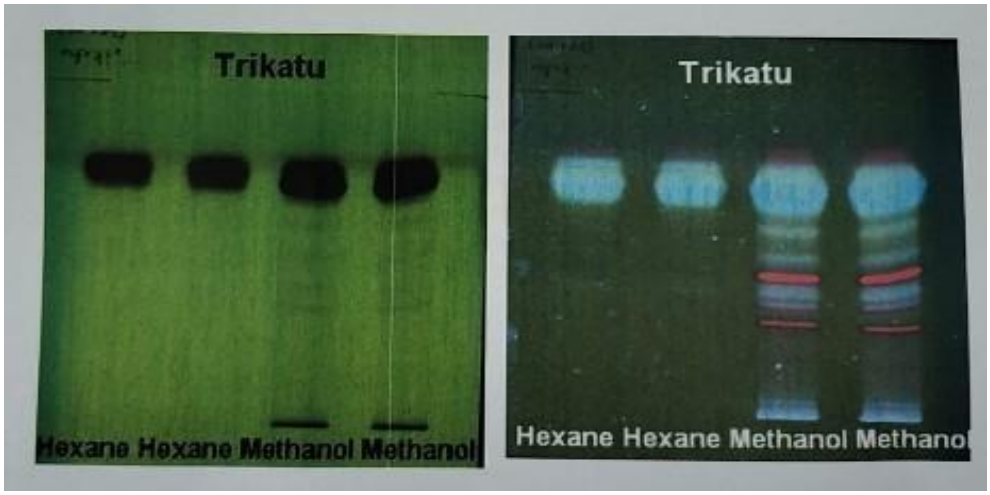
@254nm **@366nm**

Figure: 2 Hexane and methanol extract of Triphala



@254nm **@366nm**

Figure : 3 Aqueous extract of Trikatu



@254nm **@366nm**

Figure: 4 Hexane and methanol extract of Trikatu

Conclusion

The results obtained in the present study indicate Triphala and Trikatu different extracts namely aqueous, methanol, hexane and acetone are rich source of secondary metabolites as most of the class of compounds were found to be present in all these three plant parts? These findings indicate the presence of various phytochemicals in Triphala and Trikatu may be responsible for its pharmacological activities. However, there is a need to further carry out advanced studies to isolate and identify the pure active chemical compounds, and elucidate the structure of these compounds. Furthermore, these data may be handy in probing of biochemistry of this plant in the future.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Chauhan, A.; Semwal, D.K.; Misra, S.P. and Semwal, R.B. Ayurvedic research and methedology: present status and future strategies. *Ayu.*, 2015, Oct-Dec. $p_{p.}$ 364-369.

2. Jaqui, W. Herbal products are often contaminated, study finds. *BMJ*., 2013.

3. Rajendra, S. S.; Santhi, N. and Jennifer, A. HPTLC Method devlopment and Validation for Standarization of Ayurvedic Formulation, Trikatu Churna. *International Journal of Pharmaceutical and Application (UPRA),* November, 2021, Volume 6, Issue 6, $p_{p.}$379-385.

4. Baliga, M.S. et.al. Scientific validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: A review. *Chin. J. Integr. Med*., 2012, $p_{p.}$946-954.

5. Kumar, Neethu S.; Nair, Arun S.; Murali, Megha and Devi, Sreeja P.S. Qualitative phytochemical analysis of triphala extracts. *Journal of pharmacognosy and phytochemistry,* April, 2017, $p_{p.}$248-251

6. Sofowora, A. J. Complement. Med,. *Altern.* 1996, 2:365-372

7. Harbone, J. B. Phytochemical method; A guide to modern technique of plant analysis. *Chapman and Hall*, New York, 1998, 3 Edition,.

8. Kokate, C. K. A texy book of practical pharmacognosy. 5th Edition, vallabh prakashan, New Delhi, 2005, $p_{p.}$ 107-111.

9. Mehta, S.; Singh, P.S. and Saklani, P. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. *Int. J. Pharmacogn. Phytochem. Res*., 2017, 9:523-527. [Google Scholar]

10. Mukherjee, P.K. Quality control of herbal drugs: an approach to evaluation of botanicals. India: Business Horizons Pharmaceutical Publishers, 2008 [Google Scholar].

Phytochemical and antibacterial screening of leaves and latex of *Calotropis procera:* A comparison

Tripti Joshi, *Neetu Pandey
Department of Applied Chemistry and Basic Sciences
Sardar Bhagwan Singh University Balawala, Dehradun Uttarakhand, India
Email: neetu_bhtt@yahoo.co.in*

DOI 10.51129/ujpah-2024-36-1(2)

Received –27 May, 2024
Revised – 31 May, 2024
Accepted – 3 June 2024
Published –29 June, 2024

Abstract- The objective is to compare and investigate the antibacterial and phytochemical screening of leaves and latex extract of *Calotropis procera.* Extract of petroleum ether, ethanol, chloroform and water was made and all the mentioned extract were then evaluated for the phytochemical and the antibacterial activity. The antibacterial activity of leaf and latex extract has been reported with three bacteria namely *Escheria coli, S. aureus, B. cereus*. The results revealed that the best extraction solvent for antibacterial activity of leaf and latex extract is of alcohol followed by chloroform and water. The results revealed that the latex extract of *Calotropis procera* has better antibacterial effect as compare to the leaf extract of *Calotropis procera.*

Keywords: *Antibacterial, Phyto-chemical, Screening, Evaluated*

Introduction

India has been known for use of herbal drugs of the plant origin since the ancient past. Looking back at times, we could find the people have been much dependent on herbs as they are beneficial. The home remedies and DIY have been the most common and favourite way of dealing with diseases for us as we've practised this since ages and now it has been incorporated in our genes to search for these ingredients in our kitchen or in the nature. Research has explored the nature of the secondary metabolites of various medicinal plants. Medicinal plants are precious and renewable source for new drugs. W.H.O. has claimed that 80% of the world's population rely on the herbal method of treatment of various diseases. There are about 500,000 plant spices which have estimated but only a small amount has been investigated phyto-chemically. More than 130 drugs in the world's market come from higher plants either directly or synthetically[1–5]. Although hundreds of plants were tested for antifungal and antibacterial properties, the majority of them have not been adequately evaluated and processed well[6].

The whole Himalayan belt is the home of several medicinal plants not facts says so but always the science has given approval to this very fact. Himalayan Region with background information on their family, habit and nativity. A total of 190 invasive alien species under 112 genera, belonging to 47 families have been recorded. Among these, the dicotyledons represent by 40 families, 95 genera and 170 species;

monocotyledons represent 7 families, 17 genera and 20 species. The analysis of invasive species reveals that 18 species have been introduced intentionally, while the remaining species established unintentionally through trade[7].

Calotropis procera is a plant of Asclepiadaceae family and it is a large broadleaf evergreen plant with a strong odour, abundant in the tropical regions of Asia and Africa, which is commonly known as Milkweed Apple and many other names. *Calotropis procera* is used as a folk medicine and is not a new name in Indian household as it is used as ornamental plant due to beautiful white flower. It has been reported that the plant possesses potential antimicrobial, anthelmintic, anti-inflammatory, anticancer, purgative, anticoagulant, analgesic, and antipyretic characteristics and is also used in the treatment of leucoderma, leprosy, liver and abdomen diseases[8]. The latex of *Calotropis procera* has been known for important indigenous medicinal uses due to its laxative, antisyphilitic and analgesic action[9]. *Calotropis procera* flowers causes temporary paralysis of red stomach worm in sheep and notably reduces egg count percent of gastrointestinal nematodesin naturally infected sheep[10]. Dry latex of *Calotropis procera* has potential anti-cancer properties due to its differentiable targets and non-interference with regular pathway of apoptosis [11]. The pharmacological properties of *Calotropis procera* is a versatile plant for the pharmaceutical industry to develop new drugs[12]. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide[13]. The main objective of this research work is to analyze the various solvent extracts obtained from the leaf, seed and stems bark of *Calotropis procera* and to qualitatively screen them for phytochemicals using standard tests. Successful extraction, determination and isolation of biologically active components from plant material are largely dependent on the type of solvent[14].

Material and Methods

Collection: The leaves and latex of *Calotropis procera* has been collected from local areas of Balawala, Dehradun. The plant is identified at that place by the means of standard key and description.

Preparation of plant extract: Leaves of *Calotropis procera* were dried in shade to avoid direct contact of sunlight and pulverization method is used which is as follows. The dried leaves were macerated in the liquid such as hexane, isopropyl, ethyl acetate, ethanol, methanol, acetone for 48 hours. The latex was collected in sterile plastic/glass bottle by squeezing the apex and tips of leaves and kept in refrigerator at 4 ˚Celsius[15].

The latex was then dried under shade at ambient temperature with the yield of 20 gram/100 ml. To remove the chlorophyll content, the sample was extracted with petroleum ether. 20 ml of latex then further was extracted with petroleum ether in the separating funnel after the formation of two separate layers of petroleum ether and residue, the same is repeated with other solvents also.

Test organism- To study antibacterial effect of the very plant, three different bacteria's were taken namely, *Escheria*

coli, S. aureus, B.cereus from the department.

Photo-chemical Screening[16-25]

1. Test for carbohydrates

Molisch's test: Take 2-3 ml of extract and added few drops of 95% naphthol solution in alcohol. After shaking, conc. H_2SO_4, was added from the sides of the test tubes. Appearances of violet ring at the junction of two layers indicate the positive test for reducing sugar.

Benedict's solution test: Equal volume of Benedict's reagent and extract were mixed in the test tube. Heat it in boiling water bath for 5 mins. Appearance of red coloured solution indicates the positive test for reducing sugar.

2. Test for alkaloids

Dried extract was dissolved in dilute HCl. Filtered and subjected the filtrate to the following tests.

Test with Dragendorff's reagent: Took 2-3 ml of filtrate added few drops of Dragendorff's reagent. Formation of orange brown precipitates reveals the positive test for alkaloids.

Test with Mayer's reagent: Took 2-3 ml filtratered, add few drops of Mayer's reagent. Formation of cream coloured precipitates reveals the positive test for alkaloids.

Test for Hager's reagent: Took 2-3 ml of filtrate, add few drops of Hager reagent. Formation of yellow coloured precipitates revealed the positive test for alkaloids.

3.Test for proteins and amino acids

Biuret test: Took 2-3 ml of aqueous extract added 4% NaOH and few drops of 1% CuSO4 solution. Violet or pink colour was formed, proteins are present.

Ninhydrin solution test: Heated 3 ml of extract and 3 drops of 5% ninhydrin solution in boiling water bath for 10 mins. The development of violet colour showed the presence of amino acid.

4. Tests for steroids

Liebermann-Burchard reaction: Mixed 2 ml of extract with chloroform. Added 1-2 ml of acetic anhydride and 2 drops of conc, sulphuric acid from the sides of test tubes. Development of green colour revealed the positive test for the steroid.

Salkowaski reaction: Took 2 ml of extract, 2ml of chloroform and 2ml conc. sulphuric acid. After shaking appearance of red colour in chloroform layer and greenish yellow fluorescence in acid layer revealed the positive test for steroid moiety.

5. Test for flavonoids

Shinoda's test: Took 2ml of extract, 2ml ethanol, few drops of conc. HCI and little amount of magnesium turning. Appearance of pink colour revealed the positive test for flavonoids.

Lead acetate solution test: Took small quantity of extract added lead acetate solution. Appearance of yellow colour precipitate revealed the positive test for flavonoids.

6. Test for glycosides

Bontrager's test: Took 2-3 ml of extract, added dilute H_2SO_4 boiled it and filtered, then added equal volume of chloroform to appearance of red colour in ammonia layer revealed the positive test for anthraquinone glycosides.

filtrate. After shaking chloroform layer was separated.

Result and Discussion

Antibacterial test[26]

Antibacterial activity of chloroform, ethanol, aqueous extract o*f Calotropis procera* were determined by Agar well diffusion method.

Phytochemistry[27]

When the leaf and latex extract of petroleum ether,chloroform,, ethanol, and water was analysed or tested for the presence of alkaloids, flavonoids, saponins, tannins, stair instance glycoside, carbohydrate and amino acids, it was found that the different extracts showed positive test for the certain constituent as shown in Table-1and 2

When the yield of extract of leaf was observed, it was found that the maximum yield was found in the water extract that is about 7.55%, followed by ethanol 6.15%.The minimum amount of extract was formed by the petroleum ether that is 1.31%.

When ethanol, chloroform and aqueous extracts of *Calotropis procera*`s leaf was subjected to the phytochemical test, the results observed were that the petroleum ether extract was rich in glycoside and amino acid whereas ethanol and chloroform extracts were rich in tannins, carbohydrates, reducing sugar, alkaloids and saponins also some alkaloids found abundantly in aqueous extract.

Table-1 Phytochemical screening of *Calotropis procera* leaf extract

Ingredient	**Ethanol**	**Pet. ether**	**Aqueous**	**Chloroform**
%Yield	1.66	0.87	0.32	0.56
Reducing sugar	++	+	++	-
Tannins	+++	+	++	+
Steroid glycoside	++	-	+++	+
Resin	_	+	+	+
Alkaliods	_	+	_	_
Saponins	+	+	+	+
Flavonoids	++	+	++	+

+ = Present - = Absent

Table-2 Phytochemical screening of *Calotropis procera* latex extract

Solvent	**%yield**	**Alkaloids**	**Flavonoids**	**Saponins**	**Tannin**	**Glycoside**	**Amino acid**	**Carbohydrate**
Chloroform	2.1	+	_	++	+	_	_	++
Ethanol	6.15	+++	_	+++	+	++	_	+
Pet.Ether	1.31	_	_	_	_	+	+	_
Water	7.55	+	-	-	+	+	-	+

+ = Present - = Absent

From the table-2, it has been observed that the ethanolic extract of the latex was obtained in maximum amount i.e 1.66% followed by Pet.ether (0.87%).The minimum % yield was obtained in aqueous extract i.e 0.32%.

When the latex extract of *Calotropis procera* was subjected to phytochemical investigation of mainly ethanol, chloroform and aqueous extract, it was found that the ethanol extract was rich in reducing sugar, tannins, steroids, flavonoids and loaded with saponins whereas chloroform is mainly rich in tannins and saponins. Aqueous extract has tannins, steroids, resin, saponins and flavonoids[27, 28].

Zone of inhibition

The Zone of inhibition (*S.aureus)* bacteria petrolum ether extract of leaves and latex of calotrapic procera .It are shown in (Table-3) the concentration that we took were 50 mg per ml and 100 mg per ml of solvent extract for *S.aureus* bacteria only. It has been observed that the maximum zone of inhibition was found for the ethanol followed by petroleum ether latex extract when taken in 100 mg/ml concentration, followed by chloroform leaf extract when taken in 100mg per ml concentration which was 13mm, 11mm and 10mm respectively. When the results were compared to the standard that is chloramphenicol it has been observed that it showed a zone of inhibition of 15 mm for petroleum ether extract and 19 mm for ethanol where as it showed 15 mm of zone of inhibition for chloroform extract.

Similarly when zone of inhibition was observed for the bacteria *Bacillus cereus*(Table-4) the different extract of solvent of leaf and latex were treated in different concentration for the bacteria, the zone that has been observed for *Bacillus cereus* bacteria is very less as compared to *S.aureus* the maximum zone of inhibition has been shown by the petroleum ether latex extract when taken in 50 mg per ml concentration that is 6 mm whereas the ethanol extract in 50 mg/ml showed of zone of inhibition of 5 mm. Whereas the petroleum ether latex extract when taken in 20 mg/ml concentration it showed zone of 4 mm other than that chloroform and petroleum ether leaf extract showed, zone of negative, the standard chloramphenicol showed zone of inhibition of 17 mm, 15 mm and 14 mm for petroleum ether, ethanol and chloroform respectively.

When the zone was observed for *E.coli* bacteria, (Table-5) extracts were taken in the concentration of 20 mg/ml and 50 mg per litre, the maximum zone was found for ethanol leaf extract that is 6 mm at the concentration of 50mg/ml and ethanol leaf extract that is 5 mm for 50 mg per ml concentration. The chloroform extract of latex and leaf, when taken in 20mg/ml concentration showed no zone of inhibition. It has been seen that extract of *Calotropis procera* is less effective against *E.coli* bacteria by observation.

The *S. aureus* showed zone of 11mm for both leave and latex ethanol extract, whereas the water extract for leaf and latex showed a zone of 11 and 12 mm while chloroform leaf extracts showed zero zone of inhibition while latex shoes 10 mm of zone. Chloramphenicol showed zone of 14 mm when taken in 25 mg/ ml[29].

The Zone of inhibition for *Bacillus cereus* when recorded by others was found that the zone of aqueous extract of leaf when

taken in 30 mg/ml concentration was found to be 14.32mm and for methanol it was 18.24mm[30].

Similarly for *E.coli* bacteria the ethanol showed zone of 11mm and 7mm for leaf and latex extract.Water extract showed on of 10mm and 7 mm for both leaf and latex while the standard showed are zone of 13 mm[29]

Table-3 Zone of Inhibition (Bacteria - S.aureus) - Petroleum ether extract of Leaves and Latex of *Calotropis procera*

	Bacteria - S.aureus											
	Pet.ether latex		**Pet.ether leaf**		**Ethanol latex**		**Ethanol leaf**		**Chloroform latex**		**Chloroform leaf**	
Con.(mg/ml)	50	100	50	100	50	100	50	100	50	100	50	100
ZOI in (mm)	-ve	11	4	5	5	8	-ve	8	2	6	7	10
Chloroamphenicol 25mg/ml	15mm				19mm				15mm			

Table-4 Zone of Inhibition (Bacteria - Bacillus cereus) – Petroleum ether, chloroform and ethanol extract of Leaves and Latex of *Calotropis procera*

	Bacteria - Bacillus cereus											
	Pet.ether latex		**Pet.ether leaf**		**Ethanol Latex**		**Ethanol leaf**		**Chloroform latex**		**Chloroform Leaf**	
Con.(mg/ml)	20	50	20	50	20	50	20	50	20	50	20	50
ZOI in (mm)	4	6	-ve	3	2	5	1	2	1	2	-ve	1
Chloroamphenicol 25mg/ml	17mm				15mm				14mm			

Table-5 Zone of Inhibition (Bacteria – E. coli) - Petroleum ether, chloroform and ethanolextract of Leaves and Latex of *Calotropis procera*

	Bacteria - E.coli											
	Pet.ether latex		**Pet.ether leaf**		**Ethanol Latex**		**Ethanol leaf**		**Chloroform latex**		**Chloroform Leaf**	
Con.(mg/ml)	20	50	20	50	20	50	20	50	20	50	20	50
ZOI in (mm)	1	4	-ve	2	3	5	2	6	-ve	5	-ve	1
Chloroamphenicol25mg/ml	15mm				17mm				13mm			

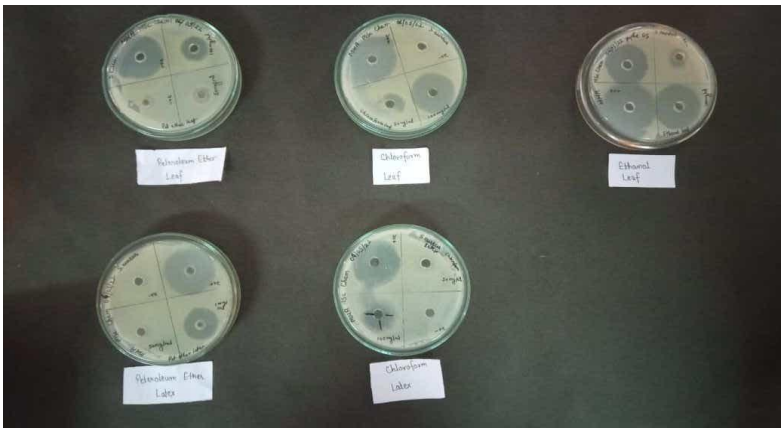


Figure-1 Zone of Inhibition for *Staphylococcus aureus*

Figure-2 Zone of inhibition for *Bacillus cereus*

Figure-3 Zone of Inhibition for *E. Coli* bacteria

Conclusion

80 grams of leaves were taken for the practical which were macerated for 48 hours in the respective solvent to get the extract of the solvent,which were used according to the polarity .The percentage yield of leaf extract of petroleum ether was found to be 1.31%, the alcohol was 6.15 %, chloroform was 2.1%, water extract was 7.5%.Water content has the maximum percentage yield.About 40 ml of latex is used and the percentage yield was found maximum for ethanol equals to 1.66%, followed by petroleum ether extract equals to 0.87%.

The zone of inhibition for *Staphylococcus aureus* for petroleum ether latex extract was shown as that 50 mg/ml, give no zone of inhibition whereas 100 mg/ml of Petroleum ether extract give a zone of inhibition of 11 mm, whereas when petroleum leaf extract was taken in the concentration of 50 mg per ml and 100 mg per ml, they give only zone of inhibition of 4 and 5 mm respectively .The ethanol latex and ethanol leave extract of 50 mg per ml give a zone of inhibition of 5 and 7 mm while when taken in 100 mg per ml it gives the zone of inhibition of 8 mm and 10 mm respectively. Chloroform latex extract when taken in 50 mg per ml gives a zone of inhibition of 2 mm, while when it is taken in 100 mg per ml it give zone of inhibition of 6 mm. Chloroform leaf extract when taken in 50 mg per ml gives a zone of inhibition of 7mm and while the 100 mg per ml concentration gives the zone of exhibition of 10 mm.

For the *Bacillus cereus* bacteria it has been found that petroleum ether latex extract when taken in concentration of 20 mg per ml and 50 mg per ml gives a zone of 4 mm and 6 mm respectively, while when petroleum ether extract of leave was taken in the concentration 50 mg/ml, gives the zone of 2 mm and 3 mm ,whereas ethanol latex extra when taken in 20 and 50 mg per zone of exhibition of 2mm and 5mm respectively ,whereas for leaf it is negative and 2 mm for 20mg/ml and 50 mg/ml respectively. For chloroform extract of latex when taken in 20 mg per ml gives 1mm of zone of inhibition while when taken in 50 mg per ml give 1 mm as zone of inhibition.

For *Escherichia coli*, bacteria when zone of any inhibition was observed for petroleum ether latex when taken in concentration of 20 mg per ml and 50 mg per ml was 4 and 6 mm respectively, whereas for petroleum ether leaf extract, it was found to be 2 mm and 8 mm for 20 mg/ ml and 50 mg/ ml respectively. The ethanol latex and ethanol leaf extract for 20 mg per ml and 50 mg per ml is 5 and 2 for 20 mg, 2 and 6 for 50 mg per ml respectively. The chloroform latex extract for 20 mg/ml shows no zone of inhibition whereas 50 mg /ml shows a 5 mm zone of inhibition, as the latex when taken in 20mg/ml gives no zone of inhibition whereas when the amount is increased upto 50 mg/ml, it gives a zone of 1mm.

From the above results, we can finally conclude that the *S.aureus* bacteria stated showing zones at a concentration higher than 20mg/ml whereas for the other 2 bacteria smaller concentration was good enough to get a zone .Hence, we can conclude that for *S.aureus* the smaller concentration of leaf and latex was not enough for antibacterial effect.

When the *B.cereus* and E.coli were observed for antibacterial activity it has been found that smaller concentration is

effective for antibacterial activity as it started showing zone in 20mg/ml concentration. It can be concluded from the above result that the leaf has greater antibacterial activity as it shows zone greater for the same concentration of latex.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

Reference

1. Borris, R.P.; Natural products research: Perspectives from a major pharmaceutical company, J. Ethno-pharmacol., 1996, 51: 29–38.
2. Kong, J.M; Goh, *N.K;* Chia L.S; and Chia, T.F; Recent advances in traditional plant drugs and orchids. *Acta Pharmacol Sin*, Morsy et al. / Phytochemical analysis of Calotropis procera, 2003, 273:7–21. N.
3. Rates, S.M.K. Plants as source of drugs. *Toxicon*, 2001, 39:603–613.
4. A. Gurib-Fakim, Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol Aspects Med*., 2006, 27: 1–93.
5. Newman, D.J; Cragg G.M and Snader, K.M. The influence of natural products upon drug discovery. *Nat Prod Res.*, 2000,17:215–234.
6. Balandrin, M.F.; Klocke, J.A.; Wurtele E.S. and Bollinger, W.H. Natural plant chemicals: Sources of industrial and medicinal materials. *Science*, 1985, 228:1154–1160
7. Jain, S.C.; Sharma, R.; Jain R. and *Sharma, R.A.* Antimicrobial activity of *Calotropis procera. Fitoterapia.*, 1996, 67:275–277.
8. Iqbal, Z.; Lateef, M.; Jabbar, A.; Muhammad G. and Khan, M.N. Anthelmintic activity of *Calotropis procera* (Ait.) Ait. F. flowers in sheep. *J. Ethnopharmacol.*, 2005, 102:256–277.
9. Larhsini, M.; Bousaid, M.; Lazrek, H.B.; Jana, M. and Amarouch, H. Evaluation of antifungal and molluscicidal properties of extracts of Calotropis procera. *Fitoterapia.*, 1997, 68:371–373.
10. Choedon, T.; Mathan G.; Arya, S.; Kumar, V.L. and Kumar, V. Anticancer and cytotoxic properties of the latex of Calotropis procera in a transgenic mouse model of hepatocellular carcinoma. *World J. Gastroenterol.*, 2006, 12:2517–2522.
11. Silva, M.C.C.; Silva, A.B.; Teixeira, F.M.; Sousa, P.C.P.; Rondon R.M.M. and Junior, J.E.R.H. Therapeutic and ´ biological activities of Calotropis procera (Ait.). *Asian. Pac. J. Trop. Med*., 2010, 3:332–336.

12. Goyal, M. and Mathur, R. Anti-microbial potential and Phytochemical analysis of plant extracts of Calotropis procera. *Int. J. of Drug Discovery and Herbal Research*, 2011, 1(3):138-143.
13. Tiwari, P.; Kumar, B.; Kaur, M.; Kaur, G. and Kaur, H. *Int. Pharm. Sciencia*., 2011, 1:98-106.
14. Verastegui, M.A.; Sanchez, C. A.; Heredia, N.L. and Santos, G.A.J. Antimicrobial activity of ectracts of three major plants from the Chauhan desert. *J. Ethnopharmacol.*, 1996, 52:1 75-177.
15. Doshi, H.; Satodiya, H.; Thakur M.C.; Parabia, F. and Khan, A. Phytochemical Screening and Biological Activity of Calotropis procera (Ait). R. Br. (Asclepiadaceae) Against Selected Bacteria and Anopheles stephansi Larvae. *Int. J. Plant Res.*, 2011, 1:29–33.
16. Mossa, J.S.; Tariq, M.; Moshin, A.; Angeel, A.M.; Al-Yahya, M.A.; Al-Said, M.S. and Rafatullah, S. Pharmacological studies on aerial parts of Calotropis procera. *Am. J. Chin. Mesd.*,1991, 3:223–231.
17. Ramaprabha, M. and Vasantha, K. Phytochemical and antibacterial activity of Calotropis procera (Ait.) R.Br. flowers. *Int. J. of Pharma and Biosci*, 2012, 3:1–6.
18. Mainsara, M.M.; Aliero, B.L.; Aliero A. A. and Yakubu, M. Phytochemical and Antibacterial properties of root and leaf extracts of Calotropis procera. *NJBAS,* 2012, 20:1–6.
19. Mohsin, A.; Shah, A.H.; Alaha, M.A.; Tariqi M.O. and Ageel, Analytic anti-pyretic activity and phytochemical.
20. Farnsworth, N.R. Biological and phytochemical screening of plants. *J pharm Sci*. 1996, 55:225–276.
21. Harborne, J.B. Phytochemical Methods, A guide to modern techniques of plant analysis. *Chapman and Hall: New York,* 1998, $p_{p.}$1–150.
22. Sofowora, A. Medicinal plants and traditional medicine in Africa, Spectrum books limited: *Ibadan. Nigeria*, 1993, $p_{p.}$ 150–289.
23. Brain, K.R. and Tuner, T.D. The practical evaluation of phyto-pharmaceuticals. *Wright Scientectica Publishers: Bristol.*, 1975, $p_{p.}$ 57–58.
24. Trease, G.E. and Evans, W.C. A textbook of pharmacognosy. *Academic press: London*, 1989, $p_{p.}$ 22–40.G.E
25. Jorgensen, J.H.; Turnidge, J.D. and Washington, J.A. Antibacterial susceptibility tests: Dilution and disk diffusion methods. In: Manual of clinical microbiology, Washington, DC: *ASM Press*,1999, 7: 1526–1543.
26. Abegunde, S.M. and Ayodele-Oduola, R.O. Department of Science Technology. The Federal Polytechnic, P.M.B. 5351, *Ado Ekiti, Nigeria.*

27. Verma, Raginee; Satsangi, G.P. and Shrivastava J.N. Microbiology Laboratory, Department of Botany, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra.

28. Salem, WM.; Sayed, W F.; Haridy, M. and Hassan, N.H. Department of Botany, Faculty of Science, South Valley University, Qena, Egypt and Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

29. Toheeb, D.Y.; Wahab, O; Okunowo, R.; Afolayan, I.; Abdulakeem, R.; Agboola, H.Y.; Lukman, A. S. and Abafi, J. M. Federal polytechnic, PRE 55, Bida Niger State, 2022, 1(2):36-46.

Isolation and Identification of a Coumarin Glycoside from leaves of *Skimmia laureola.*

D. P. Pandey
Department of Chemistry, Govt. Degree College, Dehradun-248007, India
Email: pandeydp_123@rediffmail.com

DOI 10.51129/ujpah-2024-36-1(3)

Received –27 May, 2024
Revised – 30 May, 2024
Accepted –2 June 2024
Published –29 June, 2024

Abstract- The leaves of *S. laureola* were collected from Nachiketa Tal at an altitude of 2450-2500m, District Uttarkashi, Uttarakhand, India. The air-dried and powdered leaves of *S. laureola* were exhaustively defatted with light petroleum ether (60-80^0). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, and then partitioned sequentially with $CHCl_3$ and n-butanol in a separatory funnel. The butanol layer was separated and concentrated under reduced pressure to give BuOH extract. The n-butanol extract was subjected to column chromatography over Si-gel eluted with different proportion of $CHCl_3$ and MeOH afforded 6-O-β-D-glucopyranoside-2H-1-benzopyran-2-one.

Key Words: *Skimmia laureola*, n-butanol extract, coumarin, coumarin glycosides.

Introduction

Skimmia belongs to the family *Rutaceae* is a large genus of strongly scented unarmed shrubs, distributed throughout the temperate Himalayas from north to south east[1,2]. The flowers are sweetly and leaves are strongly aromatic[3]. The leaves are often used as incense and burnt near small-pox patients for their supposed curative effects. The smoke produced by burning them is said to purify the air[4]. *Skimmia* species have been reported to possess antifungal, anti-fertility, anti-platelet, and spasmolytic activity[5-7]. Phytochemical studies on *Skimia* species resulted in the isolation of flavonoids, terpenoids, iridoids, coumarins, alkaloids, and some fatty esters[8-10]. From *S. laureola f*atty ester, terpenoids and quinoline alkaloids have been reported so far[11-14]. The present study deals with the isolation and identification of a new coumarin glycoside; 6-O-β-D-gluco pyranoside-2H-1-benzopyran-2-one from n-butanol extract of air dried leaves of *S. laureola*. Identification of these compounds was made by the concerted use of 1D and 2D spectral data.

Material and Methods

Plant Material

The leaves of *S. laureola* were collected from Nachiketa Tal (at an altitude of 2450-2500m), District Uttarkashi, Uttarkhand, India (Garhwal Himalaya) in September 2017. The plant species were identified by Dr. Jai Laxmi Rawat Department of Botany, RCU Govt. PG College Uttarkashi, Uttarakhand. A Voucher specimen (DOC 12/2009) was deposited

in the Department of Botany, Govt. P.G. College, Uttarkashi, Uttarakhand, India.

Extraction and Isolation

The air-dried and powdered leaves (3 kg) of *S. laureola* were exhaustively defatted with light petroleum ether (60-80^0). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and was washed with diethyl ether for several times. A suspension of the ethanol residue was made with water which was first partitioned with $CHCl_3$:H_2O:MeOH (6:4:4) and then with H_2O:n-BuOH; 1:1 in a separatory funnel. The n-butanol layer was separated and concentrated under reduced pressure to give n-BuOH extract. The n-butanol extract (12g) was subjected to column chromatography over S-gel eluted with $CHCl_3$:MeOH (100:0→1:1) afforded various fractions. The various fractions obtained with $CHCl_3$:MeOH (9:1) and (17:3) were mixed together and dried to get two fractions. Fraction **I** was again subjected to repeated CC over Si-gel eluted with $CHCl_3$:MeOH (9:1) gives various fractions. First few fractions were mixed together and subjected to CC over Si-gel eluted with $CHCl_3$:MeOH (4:1) and like fractions were collected and subjected to preparative TLC using $CHCl_3$:MeOH (3:1) which afforded compound **1**.

Results and Discussion

6-O-β-D-glucopyranoside-2H-1-benzo pyran-2-one (**1**): white amorphous solid, m.p. 197-199^{0}C; $[\alpha]_D^{25}$: +89^0 (c=0.1, $CHCl_3$); **HREI-MS:** m/z 324.0818, calculated for $C_{15}H_{16}O_8$; 324.0845; **IR (ν_{max}^{KBr}):** cm^{-1} 3455 (OH), 1722, 1622, 1463, 1326, 810 etc.; **UV (λ_{max}MeOH):** nm (log ∈) 205 (4.2), 230 (3.71), 256 (3.04), 317 (4.01); **^{1}H-NMR (400 MHz, CD_3OD):** δ 6.29, (1H, *d*, J=9.4 Hz, H-3), 7.92, (1H, *d*, J=9.4Hz, H-4), 7.07 (1H, *d*, *J*= 2.5 Hz, H-5, 7.15 (H-7, *dd*, J = 8.4, 2.7 Hz, H-7), 7.61 (1H, *d*, *J* = 8.4 Hz, H-8), 5.05 (1H, *d*, *J*=7.8 Hz, H-1'), 3.61 (1H, m, H-2'), 3.60 (1H, *m*, H-3'), 3.53 (1H, *m*, H-4'), 3.57 (1H, *m*,H-5'), 4.02 (1H, *dd*, *J* = 2.1, 9.4 Hz, H-6'a), 3.85 (1H, *dd*, *J* = 6.3, 9.4 Hz, H6'b) (Table-1).

^{13}C-NMR (100 MHz, CD_3OD): δ 162.82^s (C-2), 113.83^d (C-3), 144.81^d (C-4)), 115.65^d (C-5), 155.42^s (C-6), 121.83^d (C-7), 117.81^d (C-8), 149.66^s (C-9), 123.68^s (C-10), 101.35^d (C-1'), 73.91^d (c-2'), 78.10^d (C-3'), 70.77^d (C-4'), 76.83^d (C-5'), 62.25^t (C-6'). (Multiplicity of signals is given by DEPT spectroscopy, Table-1).

Acid Hydrolysis of Compound 1: 5 mg of compound **1** was refluxed with 5% aqueous HCl (5 ml) at 80°C for 3 h. After cooling, the reaction mixture was neutralized with $AgNO_3$. The aqueous layer after concentration under reduced pressure was subjected to PC using BuOH:AcOH:H_2O (5:1:4) with authentic sugars. The sugar was identified as D-glucose.

The molecular formula of compound **1** was determined to be $C_{15}H_{16}O_8$ by high resolution EI-mass spectrum which showed a quasi-molecular ion peak at m/z 324.1618. Its UV spectrum displayed absorption maxima at 205, 230, 256 and 317 nm indicating the presence of coumarin skeleton[15]. The IR spectrum displayed absorption band at 3455 cm^{-1} for OH, 1722 cm^{-1} for six membered lactone carbonyl carbon, and 1622 cm^{-1} for olifinic carbon. The ^{1}H-NMR spectrum of **1** displayed signals for 16 protons and ^{13}C-spectrum displayed presence of fifteen

carbons. The DEPT spectrum revealed the presence of four quaternary, 10 methine, and one methylene carbon atoms in the molecule. Assignment of all protons and carbon atoms were made by ^{1}H-^{1}H COSY, HSQC and HMBC spectral data [Table-1].

The ^{1}H-NMR spectrum of **1** showed two doublets (J = 9.4 Hz), each for one proton, at δ 6.29, and 7.92 which were assigned for H-3 and H-4 protons of the α,β-unsaturated lactone ring of coumarin [16]. In aromatic region presence of three signals, each for one proton, at δ 7.07 (*d, J* = 2.5, Hz, H-5), 7.15 (*dd, J* = 8.4 & 2.7 Hz, H-7) and 7.61 (*d, J* = 8.4 Hz, H-8) indicated presence of tri-substituted benzene ring in the molecule. The signal pattern of these signals clearly indicated substitution at C-6 position of benzene ring of coumarin. The ^{1}H-NMR spectrum also displayed presence of anomeric proton at δ 5.05 (d, J = 7.8 Hz) together with other sugar protons assignable to D-glucopyranoside[17]. Compound **1** on acid hydrolysis afforded sugar which was identified by PC with an authentic sugar. The β-orientation of the sugar was determined by the value of coupling constant (J = 7.8 Hz) of anomeric proton.

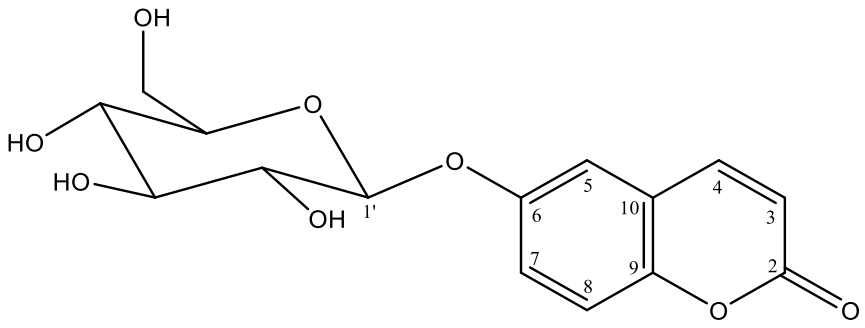
Table ^{1}H-NMR and ^{13}C-NMR data of compound **1** in CD_3OD

C/H	**$^{\delta}$C**	**$^{\delta}$H (*J* in hertz)**	**HMBC (H→→C)**
2	162.82^{s}	--	
3	113.83^{d}	6.29, *d* (9.4)	C-2, C-4, C-10 ,C-9
4	144.81^{d}	7.92, *d* (9.4)	C-2, C-5, C-8, C-9
5	115.65^{d}	7.07, *d* (2.5)	C-4, C-7, C-9, C-10
6	155.42^{s}	--	--
7	121.83^{d}	7.15, *dd* (8.4, 2.7)	C-5, C-6, C-8, C-9
8	117.81^{d}	7.61, *d* (8.4)	C-6, C-7, C-9, C-10
9	149.66^{s}	--	--
10	123.68^{s}	--	--
1’	101.35^{d}	5.05, *d* (7.8)	C-6, C-2’, C-3’
2’	73.91^{d}	3.61, m	C-1’, C-3’
3’	78.10^{d}	3.60, *m*	C-2’
4’	70.77^{d}	3.53, *m*	C-3’, C-5’
5’	76.83^{d}	3.57, *m*	C-4’, C-6’
6’	62.25^{t}	4.02, *dd* (2.1, 9.4)	C-4, C-5
		3.85, *dd* (6.3, 9.4)	C-4’, C-3’

The ^{13}C-NMR spectrum indicated presence of 15 carbon atoms, and resembled with ^{13}C-NMR data of other known coumarins [18]. The position of glucose moiety was ascertained by ^{13}C-chemical shift of benzene ring and HMBC experiment. The up-field chemical shift of C-6 carbon at δ 155.42 indicated that glucose is attached with C-6 position, which was confirmed by HMBC which displayed long-range correlation of H-1’ (anomeric proton) with C-6 of ring A of coumarin. Other long range correlations identified by HMBC are given in table 1.

On the basis of above discussed spectral data the structure of compound **1** was assigned as a coumarin glycoside; 6-O-β-D-glucopyranoside-2H-1-benzopyran-2-

one which was first time reported from *S. laureola*.



1

Acknowledgement

Author acknowledge RCU Govt. PG College Uttarkashi for the infrastructure, constant support and encouragement, Dr. Jai Laxmi Rawat Department of Botany, RCU Govt. PG College, Uttarkashi for help in the identification of plant species, and also thankful to the MRI Centre, AIIMS, New Delhi, India for the NMR facility.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

References

1. Chopra, R. N. and Chopra, I.C. *Indigenous Drugs of India*. Bimal Kumar Dhur of Academic Publisher Kolkata, 2006, $p_{p.}$ 411-412.
2. Gaur, R. D. *Flora of District Garhwal*, Trans Media, Srinagar Garhwal, 1999, $p_{p.}$ 381.
3. Genders. R. *Scented Flora of the World* St. Martin's Press, New York 1977, $p_{p.}$ 445.
4. Singh, G. and Kachroo. P. *Forest Flora of Srinagar*. Bishen Singh Mahendra Pal Singh, Dehradun, 1976.
5. Tsai, I. L.; Lin, W. Y. T.; Che-Ming, I. T.; Doong, S. L.; Huang, M. W.; Chen, Y. Ch. and Chen, I. S. *Planta Medica.*, 2000, 66 (7):6 18.
6. Atta-Ur-Rahman; Asaad, K.; Nighat, S.; Ghayur, M.; Nabeel Mesaik, M. A.; Khan, M. R.; Gilani, A. H. and Choudhary, I. M.; *J. Enzy. Inhibi. Med. Chem.*, 2006, 21(6):703.
7. Ahmad K. F. and Sultana, N. *J. Chem. Soc. Pakistan*, 2003, 25(4):328.

8. Razdan, T.K.; Harkar, S.; Qadri, B.; Qurishi, M.A. and Khuroo, M.A. *Phytochemistry*, **27(6)**: 1988 1890-1892 .
9. Boyd, D.R. and Grundon, M.F. *Tetrahedron Letters*, 1967, 8 (28):2637-2638 .
10. Kostova, I.; Simeonov, M.; Iossifova, T.; Tappe, R.; Pardeshi, N. and Budzikiewicz, H. *Phytochemistry*, 1996, 43(3):643-648.
11. Atta-Ur-Rahman; Sultana, N.; Khan, M.R.; and Choudhary, M.I. *Nat Prod Lett*., 2002, 16(5):3 05-13.
12. Sultana, N.; and Khalid, A. *Nat Prod. Res*., 2008, 22(1):37-47.
13. Atta-Ur-Rahman; A.; Khalid, A.; Sultana, N.; Ghayur, M. N.; Mesaik, M.A.; Khan, M. R.; Gilani, A. H.; and Choudhary, M. I. *J. Enzyme Inhib. Med. Chem.*, 2006, 21(6): 703-10.
14. Pandey, D.P. *UJPAH*, Dec. 2022, Vol. II, (33):55-60.
15. Sakuri, Y., and Yaguchi, T., *Phytochemistry*, 1987, 26:217.
16. Steck, W. and Mazurek, M. *J. Nat. Prod.*, 1972, 35:418.
17. Mahato, S.B.; Ganguly, A.N. and Sahu, N.P. *Phytochemistry,* 1982, 21: 959.
18. Cussans, N. J. and Huckerby, T.N. *Tetrahedron*, 1975, **31**:2719.

Studies on bioactive compounds and antimicrobial potential of Hibiscus Plant

[*1]Shweta Tyagi, [2]Ashish Kumar, [3]I.P. Pandey, [4]Anuradha Negi

[*1,2 and 4]D.A.V.(PG) College, Muzaffarnagar, Uttar Pradesh, India

[3]Emeritus Professor, Dehradun, Uttarakhand, India

[*]Email: shewtatyagi@gmail.com

DOI 10.51129/ujpah-2024-36-1(4)

Received –27 May, 2024
Revised – 31 May, 2024
Accepted – 3 June 2024
Published –29 June, 2024

Abstract- The aim of the present study was to investigate the presence of biologically active compounds (phyto-chemicals) and antimicrobial potential in leaves and flower extracts (aqueous, ethanol and acetone) of *Hibiscus* plant against pathogenic bacterial and fungal strains. Ethanol and acetone extract shown good number of bioactive compounds compared to aqueous extracts. All the tested biological compounds like protein and amino acids, carbohydrates, proteins, glycosides, tannin, phenol, alkaloids, terpenoids, flavonoids and saponin were abundantly present in plant extracts. Antimicrobial activity of different extracts of Hibiscus rosa-sinensis were tested against bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa)* and fungal strains (*Aspergillus niger, Aspergillus flavus, Penicillium crysogenum)* Antimicrobial activity was carried out by agar disc diffusion method. The result showed that, all extract showed antifungal activity against all isolated fungal strains. The acetone extracts of flower exhibited maximum inhibitory activity against the *P.crysogenum, A. niger and A. flavus*. While the ethanol extract of leaf showed minimum inhibition zone against bacterial strains. The acetone extract gave better antimicrobial activity in comparison to other extracts. Our findings prove that, the flower of hibiscus plants have medicinal antibacterial activities and can use against bacteria.

Keywords: *Hibiscus,* Antimicrobial, Bioactive compounds.

Introduction

Plants have evolved the ability to synthesize chemical compounds that help them to defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. By chance some of these compounds whilst being toxic to plant predators turn out to have beneficial effects when used to treat human diseases. (Reena Patel *et al*, 2012). Importance of the plants basically originates due to the presence of specific biological active classes of organic compounds (Shivananda,2007). Many of the herbs and spices used by humans are useful medicinal compounds (Uddin, 2010) More than 80% world's population still emphasize to use traditional and old medicinal system such as Homeopathy, Unani, Ayurveda, Sidha, Naturopathy etc. *Hibiscus rosa-sinensis is* (hibiscus plant) is member of the family Malvaceae, grow as green herbaceous plant in tropical

regions. It is a tropical shrub, with large, glossy green leaves and spectacular trumpet shaped flowers. Its medicinal values have been mentioned in traditional folk medicines for variety of diseases. Flowers and leaves are found to possess antioxidant, antifungal, anti-infectious, antimicrobial, anti-inflammatory, anti-diarrheic and antipyretic activity (David and Leonard, 1998). Traditionally the flower can be using as anti-asthmatic agent (Zhao et al., 2010). The aim of this study is to evaluate the anti-microbial efficacy of the plant extract of *Hibiscus rosa-sinensis is* against *Penicillium crysogenum, Aspergillus niger, Aspergillus flavus, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. And* investigated the presence of biologically active compounds (phytochemicals) in extracts.

Materials and methods

Collection of plant material: The leaves and flowers of. *hibiscus* were collected from the Botanical Garden, Department of Botany, D.A.V. (PG) College, Muzaffar-nagar, UP, India. The different plant parts were washed with running tap water and subjected to crude extract preparation at room temperature for further studies.

Preparation of crude extract: The leaves and flowers of the plant *hibiscus* were used for crude extract preparations separately for phytochemical and antimicrobial analysis. 10 g (fresh weight) of different parts (leaf and flower) of *hibiscus* were homogenized in 3-5 folds of aqueous and organic solvent (50%, ethanol and acetone) in pestle and mortar at room temperature. The extracts were filtered through sterilized Whatman filter paper and filtrate was centrifuged at 10000 rpm at 4^0C for 5 minutes. The clear supernatant was used as crude extract for phytochemical analysis and antimicrobial testing. Extracts were kept at 4 °C for the further analysis.

Isolation, purification and identification of microbial strains from soil and spoiled fruits: The microbial strains were isolated from soil by serial dilution method. (Cappuccino and Sherman, 2005; Aneja , 2009) and by some spoiled fruits. The isolated colonies of *Penicillium crysogenum, Aspergillus niger and Aspergillus flavus* were streaked onto the fresh SDA plates while *E. coli, Bacillus sp. and Pseudomonas sp*. were streaked onto the fresh NAM plates.

Screening of antimicrobial activity: Antimicrobial activity of plant extract was screened by agar disc diffusion method on SDA plate for fungal strains and NAM plates for bacterial strains and measure the zone of inhibition in mm. (Khokra *et al*, 2008).

Phytochemical screening of different parts of *T. corymbosa*: Phytochemical tests were carried out in the aqueous and organic extract of *T. corymbosa* using standard methods to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1989), Omoya and Akharaiyi (2012), Jyothiprabha and Venkatachalam (2016), Harborne and Williams (2000).

Screening for Tannins: 5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One ml ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

Screening for Terpenoids: 5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening for Flavonoids: A pinch of zinc dust was added to 2 ml of extract followed by the addition of 1 ml concentrated HCl. Appearance of pink colour indicate the presence of flavonoids

Screening for Saponins: 5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence for the presence of the saponins.

Screening for Glycosides: 5 ml extract was mixed thoroughly with 1 ml of glacial acetic acid and 1 ml of 5% FeCl3 solution in a test tube. 1ml of concentrated sulphuric acid was added to the above reaction mixture carefully along the side of test tube. Development of green-blue colouration shows the presence of glycosides. (Kellar- Kiliani test).

Screening for Alkaloids: Mayer's test: 1 ml of every extract mix with a drop of Mayer's chemical agent is additional by the aspect of the test tube. A creamy or white precipitate indicates the presence of alkaloids.

Screening of Phenols: Few drops of 10% lead acetate solution were added to 5ml of test solution. Formation of white precipitates indicates the presence of phenol in the test solution.

Results and Discussion

In the present investigation bioactive substances (phytochemical) analysis has been performed in different extracts of hibiscus plant (leaves and flower) which showed the presence of various phytochemical constituents, glycosides, tannin, phenol, alkaloids, flavonoids, terpenoids, phenols and saponin (as shown in Table 1). All the phytochemicals are abundantly found in flower extract (aqueous, ethanol, acetone) followed by in leaf extract respectively. This phytochemical screening is more prominent in acetone extract and ethanol extract as compared to aqueous extract as bioactive compound are organic in nature Presence of high level of total phenols, flavonols, flavonoids, and anthocyanins has been reported in different flowers and their extracts (Cai et al., 2004; Gouveia et al., 2013; Yanget al., 2012; Wijekoon et al., 2011) and soluble in organic solvent. Presence of high level of total phenols, flavonols, flavonoids, and anthocyanins has been reported in different flowers and their extracts (Cai et al., 2004; Gouveia et al., 2013; Yanget al., 2012; Wijekoon et al., 2011)

The presence of phytochemicals as such; flavonoid, alkaloid, tannin showed cytotoxic effect (Chowdhury et al. 2017). Additionally, cholesterol-lowering, as well as cytotoxic qualities, anti-bacterial, anti-viral properties, are credited to the presence of saponin (Bailly & Vergoten, 2020). The Flavonoids and phenols are major compounds that act as antioxidants or free radical scavengers (Bhandary *et al.*, 2012). Tannin shows an anticancer property that is perceptible from its inhibitory activity towards growth while the phenolic compound, tannin, terpenoid,

flavonoids possess an ant-helmintic property so the plant Zanthoxylum, Acorus could be used to treat stomach problems (Nath & Yadav, 2016).

Table 1: Screening of bioactive compounds (phytochemicals) in leaf and flower of *Hibiscus rosa-sinensis is* in aqueous, ethanol and acetone extracts.

S.No.	Name of bioactive compound	Leaf			Flower		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	Alkaloids	+++	+	+++	+++	+++	+++
2	Glycosides	++	++	+++	+++	+++	+++
3	Tannin	+	++	+++	+++	+++	+++
4	Terpenoids	++	+++	++	+	++	+++
5	Phenols	++	+++	+++	+	++	+++
6	Saponin	+++	+++	+++	+++	+++	+++
7	Flavonoids	++	++	++	+++	+++	+++

+ = presence, - = absence, ++= moderate, +++= abundant

Table 2 showing the antimicrobial potential of *Hibiscus rosa-sinensis is* leaves and flower in aqueous, ethanol and acetone extract against isolated fungal and bacterial strains. It is observed that the flower extract prepared in acetone showed the maximum inhibition zone against isolated bacterial strains i.e. *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and fungal strains (Aspergillus niger, Aspergillus flavus, Penicillium crysogenum*). While the extract of leaf in distilled water, acetone and ethanol showed less activity against fungal strain *Aspergillus niger*, *Aspergillus flavus* and *P. crysogenum*. The minimum inhibition zone is found in distilled water leaf extract against *A. niger* while the maximum zone found against *Pseudomonas aeruginosa* in flower acetone extract. This finding in agree with the finding of (Uddin et al., 2010), reported that the methanolic extract of Hibiscus flower obtain antibacterial effect at high concentration. Polyphenols, flavonoids and tannins present in a sample might be responsible for the observed antibacterial activity. These compounds are generally produced by plants as a mode of defense against microbial infections. (Scalbert, 1991)

Table-2 Antimicrobial screening in leaf and flower of *Hibiscus rosa-sinensis is* in aqueous, ethanol and acetone extracts

S.No.	Name of Microorganism	Leaf extracts of hibiscus (Inhibition zone in mm± SD)			Flower extracts of hibiscus (Inhibition zone in mm± SD)		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	***Penicillium crysogenum***	12.2±0.21	15.1±0.25	19.3±0.22	15.5±0.21	18.4±0.31	22.2±0.31
2	***Aspergillus niger***	11.1±0.18	18.2±0.26	12.4±0.22	11.6±0.31	15.7±0.23	19.4±0.23
3	***Aspergillus flavus***	14.4±0.27	14.1±0.25	14.2±0.31	14.7±0.25	20.1±0.26	24.8±0.26
4	***Pseudomonas aeruginosa***	16.8±0.35	19.6±0.33	21.7±0.19	19.9±0.22	24.8±0.29	29.7±0.29
5	***Staphylococcus aureus***	17.3±0.35	23.5±0.24	20.1±0.23	20.2±0.21	28.4±0.34	27.5±0.25
6	***Bacillus subtilis***	18.4±0.29	21.2±0.25	22.7±0.22	18.4±0.32	25.5±0.12	28.6±0.22

Conclusions

From the study, it could be concluded that plants are a great source of phytochemicals that could be utilized in curing various ailments. The bio active substances in plants are produced as secondary metabolite i.e. Tannin, terpenoids, flavonoid, saponin, alkaloids, glycosides, phenols. This phytochemical screening test may be helpful in the screening of bioactive compound and eventually may provide a therapeutic platform to develop new drugs. The flower acetone extract of *Hibiscus* plant can be considered to be as equally potent as the most of effective artificial antibiotics.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

References

- Aneja, K.R. Experiments in microbiology, plant pathology and biotechnology, New Age International (P) Ltd. Publishers, Fourth Edition: 2003, Reprint: 2009, ISSN (10): 81-224-1494-X, ISSN (13): 978-81-224-1494-3. associated with anticancer. *Life Sciences*, 2009, 74:2157–2184.
- Bailly, C.; and Vergoten, G. Esculentosides: Insights into the potential health benefits, mechanism of action, and molecular target. *Phytomedicine*, 2020, https://doi.org/10.1016/j.phymed.2020.153343.
- Bhandary, S.K.; Sucheta, K.N.; Bhat, V.S.; Sharmila, K.P. and Bekal, M.P. Preliminary Phytochemical Screening of Various Extracts of Punica granatum Peel, Whole Fruit and Seeds. Nitte, *University Journal of Health Science*, 2012, 2(4):2249-7110
- Cai, Y.; Luo, Q.; Sun, M. and Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, 2004, 74:2157–2184.
- Cappuccino, J.G. and Sherman, N. Microbiology: a laboratory manual, 7th edition, Pearson education. Inc and Darling Kidersley (India), 2005, pp.143-203.
- Chaudhary, A.; Sharma, S.; Mittal, A.; Gupta, S. and Dua, A. Phyto-chemical and antioxidant profiling of *Ocimum sanctum*. *Journal of Food Science and technology*, 2020, 57(10):3852-3863, https://doi.org/10.1007/s13197-020- 04417-2.
- David, B. and Leonard, L.A.X. Healing plants of the Hawaiian kingdom *Hibiscus rosa-sinensis is* (Aloalo). Roast Duck Production, 2020.

- Gouveia, S.; Alves, J. and Castilho, P.C. Characterization of phenolic compounds and antioxidant activity of ethanolic extracts from flowers of *Andryala glandulosa* ssp. varia (Lowe ex DC.) R. Fern., an endemic species of Macaronesia region. *Industrial Crops and Products*, 2013, 42-573–582
- Harborne, J.B. and Williams, Ch. A. Advances in flavonoid research sinc.1992. *Phytochem*., 2000, 55:481-504.
- Jyothiprabha, V. and Venkatachalam, P. Preliminary phytochemical screening of different solvent extracts of selected lndian spices. *Int. J. Curr. Microbiol. App. Sci*., 2016, 5(2):116-122.
- Khokra, S. L.; Prakash, O.; Jain, S.; Aneja, K. R. and Dhingra, Y. Essential oil composition and antibacterial studies of *Vitex negunda* Linn. Extract, India. *J. Pharmaceutical Science*, 2008, 70:522-526.
- Nath, P. and Yadav, A. K. Anthelmintic activity of a standardized extract from the rhizomes of *Acorus Calamus* Linn. (Acoraceae) Against Experimentally Induced Cestodiasis in Rats, *J. Intercult. Ethnoparmacol*., 2016, 5(4): 390-395. https://doi.org/10.5455/jice.20160521124439, Accessed: 20.09. 2018.
- Omoya, F. O. and Akharaiyi, F. C. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. *Int. Res. J. Pharm*, 2012, 2(5):127-132.
- Phenolic compounds of 112 traditional Chinese medicinal plants
- Patel, R.; Patel, A.; Vaghasiya, D. and Nagee, A. Antimicrobial Evaluation of Hibiscus Rosa-sinensis plant extracts against Some Pathogenic Bacteria, Bulletin of Environmental and Scientific Research ISSN 2278-5205, Available online at http://www.besr.org.inVol.1, Issue (3-4), pp.14-17.
- Scalbert, A. Antimicrobial properties of tannins. *Phytochemistry*, 1991, 30: 3875–3883.
- Shivananda, N.B.; Sivachandra, R.S.; Orette, F.A. and Chalapathi. Effects of *Hibiscus rosa-sinensis is* L (Malvaceae) on wound healing activity: a preclinical study in a Sprague Dawley rat. *Int. J. Lower Extremity Wounds*, 2007, (6):76-81.
- Sofowara, A. *Medicinal plants and Traditional medicine in Africa*, 1993.
- Trease, G. E. and Evans, M. C. *Pharmacognosy*, Elsevier, 2005, 14th ed. 53:431-512.
- Uddin, B.; Hossan, T.; Paul, S.; Ahmed, T.; Nahar, T. and Ahmed, S. Antibacterial activity of the ethanol

extracts of *Hibiscus rosa-sinensis is* leaves and flowers against clinical isolates of bacteria. Bangladesh *J. Life Sci.*, 2010, 22: 65- 73.

- Wijekoon, M.M.J.O.; Bhat, R. and Karim, A. A. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bungakantan (Etlingera elatior Jack.) in florescence. *Journal of Food Composition and Analysis*, 24:615–619.

- Yang, D. J.; Chang, Y. Z.; Chen, Y. C.; Liu, S.C.; Hsu, C.H. and Lin, J. T. Antioxidant effect and active components of litchi (*Litchi chinensis Sonn*.) flower. *Food and Chemical Toxicology*, 2012, 50:3056–3061.

- Zhao, J.; Zhou, L.; Wang, J.; Shan, T.; Zhong, L. and Lix, X. Endophytic fungi for producing bioactive compounds originally from their host plant. *Curr. Res. Technol. Education Topics in Appl. Microbiol. Microbial Biotechnol.*, 2010, 1:5.

Qualitative investigation and screening of antimicrobial activity of stem extract in *Clerodendrum infortunatum* plant

[*1]Dipendra Singh, [1]Rita Saini and [1]Shivanand Patil

[1]Department of pharmacy, Shree Dev Bhoomi Institute of Education Science and Technology, Pondha, Dehradun, Uttarakhand-248007

[*]Email id: sdipendra511@gmail.com

DOI 10.51129/ujpah-2024-36-1(5)

Received – 01 June, 2024
Revised – 04 June, 2024
Accepted –08 June, 2024
Published –29 June, 2024

Abstract- *Clerodendrum infortunatum* is known as hill glory bower and a plant which is widely distributed throughout the whole world. Up to now, many species of *C. infortunatum* have been described in various indigenous systems of medicine that are used in preparation of folklore medicines for the treatment of various life-threatening diseases, and much of this plant have been very well studied for their chemical constituents and biological activities. It also is used in Unani, Ayurveda, and siddha system of medicine for many years. From *Clerodendrum infortunatum* many compounds, including mono-turpentine and its derivatives, sesquiterpene, di-terpenoids, tri-terpentine, flavonoid, quercetin, acacetin, gallic acid, sterol and flavonoid glycoside, phenylethanoid glycoside, steroids and steroid glycosides, cyclohexyleth-anoids, anthraqunine, cyanogenic glycolsides, and others have been isolated and identified. In the present study, Chloroform, pet. ether, and water stem extract of Clerodendrum infortu-natum obtained by Soxhlet extraction was screened to detect the presence or absence of several bioactive compounds which are reported to cure different diseases. Anti-microbial analysis of stem extract was carried out against *lacto-bacillus, E.coli* and *staphylococcus aureus* organisms by agar well diffusion method. It was observed that the zone was recorded against this organism. The results indicate that the chloroform, pet.ether and water extracts of *C. infortunatum* are having anti- microbial efficiency in controlling the microorganisms. So, *Clerodendrum infortunatum* is the plant which are beneficial on human health.

Keyword: *Clerodendrum infortu-natum*, qualitative investigation and anti-microbial activity.

Introduction

Clerodendrum infortunatum is a plant (Figure 1) from the Verbenaceae family that has been used as medicine in India for centuries. In Hindi and Malayalam, the plant is referred to as Peruvelam and Bhant respectively. It is frequently found in India's arid plains and lands. The therapeutic qualities in leaves, bark, roots, flowers, stems, and seeds are present. The plant's many parts have been used to cure digestive issues, anemia, malaria, inflammatory illnesses, tumors, snakebites, and more. These plants have been beneficial starting point

for the creation of drugs. Lead optimization programs are used to create safe and efficacious medicines by using phytochemicals as templates. Many active substances, including alkaloids, terpenoids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids that are deposited in their different sections, such as leaves, flowers, bark, seeds, fruits etc. (Figure 1).

Figure 1 *Clerodendrum infortunatum* **plant**

According to the World Health Organization[2] approximately 75% of people on the planet use plants as medicine for a variety of ailments.The widespread use of medicinal plants in healthcare is mostly due to a number of causes, including the adverse effects of synthetic pharmaceuticals, the high expense of industrialised medicines, and the lack of public access to pharmaceutical and medical treatment. [3]. Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi, insect[4]. Natural products have been a consistently successful source in drug discovery and offer more opportunities to find antimicrobial drugs[5].

Benefits of *clerodendrum infortunatum*

Clerodendrum infortunatum provide Health benefits, financial benefits, and society-wide benefits.

Health benefits

Medicinal plants can enhance the immune response to disease agents and a potential source of bio-molecules to treat diseases like cancer, diabetes, and hypertension. There herbs are also known for their reputation for fewer side effects.

Financial benefit

People who harvest, process, and distribute medicinal plants for sale can earn financial benefits. Local traders can also sell their products at higher prices and open new markets.

Society-wide benefit

Medicinal plants provide society wide income and livelihood for people in the area. They also play a role in strengthening health care opportunity for people with noncommunicable disease.

Aim and Objective

- To study about qualitative investigation of it plant.
- To study about preparation of stem extract by using different extract solvent.

- To create antimicrobial agent by using stem extract from *Clerodendrum infortunatum* plant.

Material and Methods

- Proper and timely collection of the *Clerodendrum infortunatum* plant
- Authentication by the expert
- Adequate drying
- Grinding
- Extraction
- MIC (Minimum Inhibitory Concentration)

Plant collection

These are the plant which are collected from majhau village, dehradun district (Uttarakhand).First the fresh stem of the collected plant were separated, wash cleanly several time using tapwater, thereafter the stem were rinsed by using distilled water, and then shade dried at 28 0C.for 72 hour. The stem dried stem were grind into a powder from this material kept in air tight bottle for other extraction[6].

Figure-2 Dried stem powder of Clerodendrum *infortunatum*

Preparation of extracts

Crude plant extracts were prepared by Soxhlet extraction method. About 35gm of powdered *Clerodendrum infortunatum* plant material was uniformly packed into a thimble and extracted with 400ml of different solvents separately. Solvents used were, Petroleum ether, Water and chloroform. The process of extraction continued for 24 hours or till the solvent in siphon tube of an extractor became colorless. After that, the extracts were taken in the beakers and kept on hot plate and heated at 30-40ºC till all the solvents got evaporated. Dried extracts were kept in refrigerator until use[7].

Figure 3 Chloroform extract.

Figure 4 Water extract.

Figure 5 pet.ether extract

Qualitative analysis of *Clero-dendrum infortunatum* plant

Stem extracts prepared in three different solvents (water, petroleum ether, chloroform) were used for experimental purpose.

Test for flavonoids

2-3 drop of sodium hydroxide were added to 2ml of petroleum ether extract, it gave deep yellow color so this solution was indicating that flavonoid was present. 2-3 drop of sodium hydroxide were added to 2ml of chloroform extract, it became colorless by adding few drop of dilute HCL it give yellow color which indicated flavonoid were present. 2-3 drop of NaOH were added 2 ml of water a yellow color appeared which indicated flavonoid was present[(8)].

Test for alkaloids

Few drops of Mayer's reagent were added to 1 mL of chloroform extract. A yellowish precipitate was formed, indicating the presence of alkaloids.1ml of water extract which added few drop of Mayer's reagent desired precipitate was formed which indicating alkaloids are present in the solution. Few drop of Meyer's reagent were added to 1ml of pet.ether it give yellow precipitate which indicate there is alkaloids present[(9)].

Test for phenol

Few drop of ferric chloride solution were added to 1ml of water extract. A dark green precipitate was formed, indicating presence of phenol. 1 ml of pet.ether extract added few drop of ferric chloride solution gave colorless precipitate which indicated there is no phenol compound. Similarly, in chloroform extract it gave greenish color which indicates that was presence of phenol.

Test for anthraquinone

In anthraquinone test, Bromine test was used. 2ml of bromine to added equal volume of pet ether extract to give pink precipitate which indicated there was presence of anthraquinone. In water and chloroform extract which do not give pink precipitate, which mean that was no presence of anthraquinone

Test for terpenoids

5ml of pet.ether, and aqueous solution mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid were carefully added was a reddish brown coloration of the interface was formed to show the presence of terpenoids.

Test for tannins

In the tannins test ferric reagent was used in this test 3 drops of ferric reagent were added to a 2ml of sample(chloroform, pet.ether, water) extract, the chloroform extract and pet.extract gave gray color which indicated the presence of tannin water doesn't gave color which indicated there is no presence of tannin.

Test for steroids

This test is given by Lieberman Burchard reaction to the chloroform solution in a test tube, few drop of acetic anhydride was added, 1ml of concentrated sulphuric acid allowed to stand a reddish ring was formed which showed there was presence of steroids in the pet.ether and the aqueous solution didn't give positive reaction.

Test for glycosides

In glucosides test at first 0.5ml of glacial acetic acid and 2-3 drop of ferric chloride

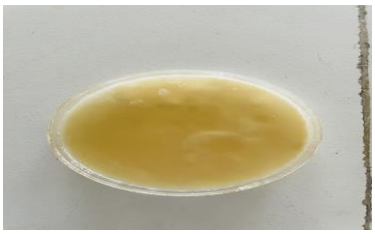
was mixed with Chloroform, pet.ether, water then 1 ml of concentrated H_2SO_4, then water and pet.ether gave deep blue color which indicated presence of glycosides.

Phytochemical analysis of steam extract

Preparation of agar media

In a beaker, 28 grams of the dehydrated powder or lab-prepared media is added to 1000 milliliters of distilled water. The suspension is then heated to boiling to dissolve the medium completely.The dissolved medium is then autoclaved at 15 lbs pressure (121°C) for 15 minutes.Once the autoclaving process is complete, the beaker is taken out and cooled to a temperature of about 40-45°C..The media is then poured into sterile Petri plates under sterile conditions. Once the media solidifies, the plates can be placed in the hot air oven at a lower heat setting for a few minutes to remove any moisture present on the plates before use[(10)].





S.No	Test	Pet.ether	Chloroform	Water
1	Flavonoids	+	+	+
2	Alkaloids	+	+	+
3	Phenol	-	+	+
4	Anthraquinone	+	-	-
5	Terpenoids	+	-	+
6	Tannins	+	+	-
7	Steroids	-	+	-
8	Glycosides	+	-	+

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The

antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested[(11)].

Anti-Microbial Activity

The chloroform, pet.ether, and water extract was prepared by using the Soxhlet apparatus of *Clerodendrum infortunatum* to study its antimicrobial potential. Antimicrobial analysis of extract was carried out against lacto-bacillus organisms. The zone of inhibition in mm for the tested organism with the Chloroform, pet.ether and water extract of *Clerodendrum infortunatum* and by agar well diffusion method. In the present study, Chloroform, pet.ether, and water, stem extract *Clerodendrum infortunatum* obtained by Soxhlet extraction was screened to detect the presence or absence of several bioactive compounds which are reported to cure different diseases. Anti-microbial analysis of stem extract was carried out against lacto-bacillus organisms by agar well diffusion method. It was observed that the zone of was recorded against lacto-bacillus organism. The results indicates that the chloroform, pet.ether and water extract of C. amboinicus is having anti- microbial efficiency in controlling the microorganisms.

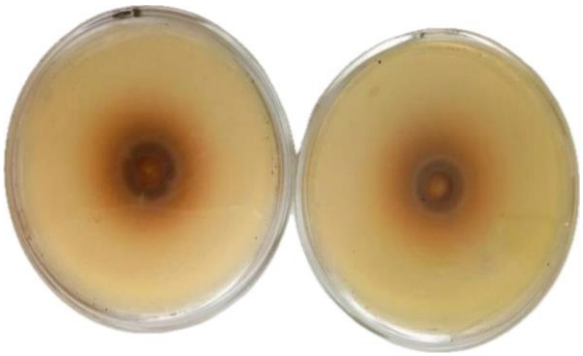


Figure-6 Zone of inhibition of Lactobacillus from Pet.ether extract (C.*infortunatum* plant)

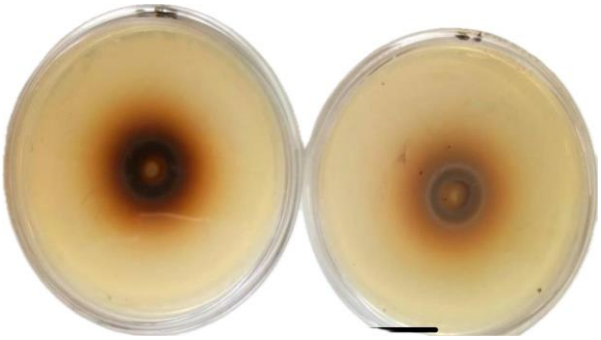
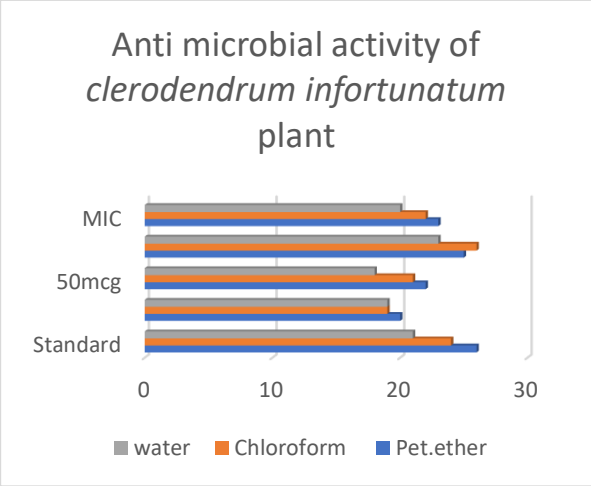


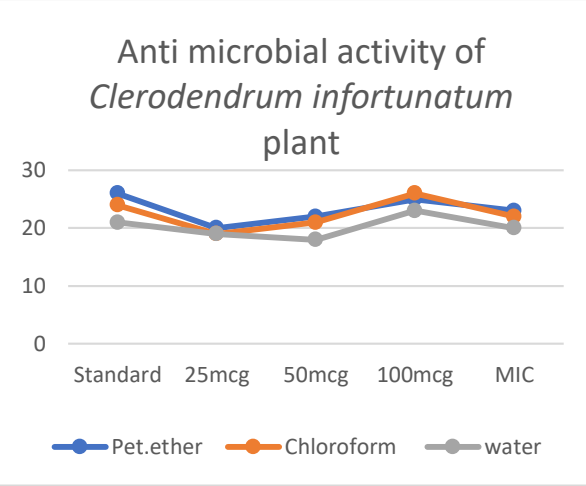
Figure-7 Zone of inhibition of Lactobacillus from chloroform extract(*C.infortunatum* Plant)

Figure-8 Zone of inhibition of lactobacillus from Water extract (*C.infortunatum* Plant)

Minimum inhibitory concentration of *Clerodendrum infortunatum* **plant**

Extract	**Standard(amoxicillin) in mm**	**25mcg**	**50mcg**	**100mcg**	**MIC**
Pet.ether	26	20	22	25	**23**
Chloroform	24	19	21	26	**22**
water	21	19	18	23	**20**





Conclusion

Numerous phytochemicals, including alkaloids, flavonoids, steroids, saponins, and tannins, were identified by the phytochemical study conducted in this paper. The phytochemical component verified the use of stem as a medical treatment. Indians today use medicinal plants and phytochemicals extensively for health treatment. *Clerodendrum infortunatum* can be identified with the help of this investigation. Investigations on the antimicrobial efficacy of Clerodendrum *infortunatum*'s water, pet.ether and chloroform extract against organisms resistant to many drugs have been conducted. The findings of the investigation demonstrate that the dried stem powder extract of *Clerodendrum infortunatum* in various solvents exhibits a strong antimicrobial action against microbes that are resistant to drugs, including lacto bacillus.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

References

1. Pawar, Mahesh. Antimicrobial activity and phytochemical study of leaf of *Clerodendrum infortunatum* medicinal plant. *Journal of emerging technologies and innovative research (JETIR)*, ESTD Year **2014,** ISSN:2349-5162, f396-f399.

2. WHO. Antimicrobial resistance: the facts. *Essent. Drugs Monit.,* 2000,28 (29): 7-19.

3. Johann, S.; Pizzolatti, M.G.; Donnici, C.L. and Resende, M.A. Antifungal properties of plants used in Brazilian traditional medicine against clinically relevant fungal pathogens. *Braz. J. Microbiol.,* 2007, 38(4):632-637.

4. Van Wyk, B.E. and Gericke, N. People's plants a guide to useful plants of southern Africa. *Pretoria: Briza publications,* 2000, PMCid:PMC176 3281.

5. Wang, R.; Gu, Q.; Yang, L.; Chen, J.; Li, S. and Zheng, Y. Anti-HIV-1 activities of extracts from the medicinal plants Rhus chinensis. *J. Ethno pharmacol*, 2006,105: 269-273.

6. Sonali, D.; Suple and Varsha, D. Hutke Secondary metabolite and antioxidant properties of root and leaf extract of *Clerodendrum infortunatum* l. *International Journal Of Creative Research,* Volume 10, Issue 1 January 2022 | ISSN: 2320-2882 page no s492-497.

7. Mahesh, A. Pawar Antimicrobial activity and phytochemical study of leaf of celerodendrum *infortunatum*. Medicinal International Journal of Creative Research, volume 10, Issue 5, May 2023, ISSN:2349-5162 $p_{p.}$ 396-399.

8. Daf, Abhijit N.; Shekhar, B.; Waikar, Akshay; Madavi, N.; Kapse, Akash S.; Pise, Shailesh B.; Sawarkar, Dhananjay G. and Mundhada, Dharmendra. Comparison of Different Solvent and Extraction Methods for Isolation of Flavonoids Compound from Leaves of *Clerodendrum infortunatum* Linn. *Journal of Pharmacognosy and Natural Products Volume*, 2022, 8:6, $p_{p.}$1-14.

9. Remya Balan, M. I. and Susha Dayanandan. Phytochemical Study of Methanolic Extract of Indian Bhant Tree, Clerodendrum *infortunatum* . *International Journal of Scientific Research and Reviews IJSRR,* 2019, 8(1):394-404.

10. Nalla Priyanka'*, M.; Srikanth', G.; Raveendra Babu', M.; Sowjanya', V.; Ramya Sri', M.; Vasanthi', K.; Sri Lakshmi Bhargavi, Y.; Tirupathaiah', K. and Mohana Sri Vasavi', N. Raviteja' In-vitro Screening of Anti-microbial, Anti-fungal and Anti-oxidant activities of Ethanolic leaf extract of Anthocephalus cadamba. *Asian Journal of Pharmaceutical Research,* 12(4): October - December, 2022, $p_{p.}$275-280.

GC-MS study and anti - microbial activity of essential oils isolated from leaves of *Artemisia annua*

[*1]S C Sati, [2]Maneesha D Sati and [3]Sushil Kumar

[*1 and 2]Department of Chemistry, HNB Garhwal University (A Central University), Srinagar (Garhwal), Uttarakhand, India,

[3]Department of Chemistry, Govt PG. College Augustymuni Rudraprayag, Garhwal, Uttarakhand, India

[*]Email: sati_2009@rediffmail.com

DOI 10.51129/ujpah-2024-36-1(6)

Received – 04 June, 2024
Revised – 08 June, 2024
Accepted –13 June, 2024
Published –29 June, 2024

Abstract- The chemical composition of the essential oil obtained from the leaves of *Artemisia annua* collected from the Garhwal region of Uttarakhand, was analyzed by GC-MS. The major constituent was found out to be (Z)-β-Farnesene (18.80%). The antibacterial and antifungal activity of the oil was determined by disc diffusion method. Results showed that the oil exhibited mild antimicrobial activity.

Keywords: *Artemisia annua Antibacterial, Antifungal, Asteraceae, Essential oil.*

Introduction

Artemisia annua L. is an annual, herbaceous, aromatic medicinal plant belonging to the family, Asteraceae. It is mentioned in traditional Chinese medicine as a cure for different diseases like fever, hemorrhoid, and malaria. It is native to the mild and temperate climate of Asia but has been naturalized to other countries outside Asia as well. The most common ethnobotanical usage of this plant involves the use of whole plant decoction for the treatment of cold, malaria, and cough. The whole flowering plant is known to be antipyretic, antihelminth, antispasmodic, antiseptic, and antimalarial. The antimalarial activity of this plant is due to artemisinin, a sesquiterpene lactone containing an endoperoxide moiety that acts as a key pharmacore[(1)]. Artemisinin forms an important part of combinatorial treatment therapy recommended for the treatment of malaria. Artemisinin and its derivatives like artesunate have also been reported to have potent anticancer properties as well. Besides artemisinin, certain other phytochemicals reported in this plant are monoterpenes, poly-phenols, coumarins, flavones, flavonols, phenolic acids, many sesquiterpenes properties. They have been reported to synergize the activity of artemisinin and its derivatives against malaria[(1)]. Considering the immense medicinal properties of this plant, it was selected for present investigations, The present paper deals with GC MS analysis and study of antimicrobial activity of the plant.

Material and Methods

Plants Materials

Whole plants of *Artemisia annua* was collected from the Bugani road, Srinagar Garhwal, Uttarakhand, India. The plant was identified from Department of Botany, HNB Garhwal University Srinagar, Uttarakhand. A Voucher Specimen (GUH-3354) was deposited in the Department of Botany.

About 10 kg sample of dried leaves of *Artemisia annua* were subjected to hydro distillation for 8 hours using a Clevenger-type apparatus. The oil was extracted over ether and dried over anhydrous Na_2 SO_4. The yield was 0.05% (v/w).

GC and GC/MS

GC/MS analysis were performed with a Perkin Elmer Clarus 500 gas chromatograph equipped with a split/splitless injector (split ratio 50:1) data handling system. The column was Rtx-5 capillary columns (60 m x 0.32 mm, 0.25 μm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 mL/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI+ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as follows: initial temperature of 600C (hold: 2 min) programmed at a rate of 3^{0}C /min to a final temperature of 2200C (hold: 5 min). The temperatures of the injector, transfer line and ion source were maintained at 210^{0}C, 210^{0}C and 200^{0}C, respectively.

Identification of Compounds

The components of the oils were identified by comparison of their mass spectra with those of computer library (NIST/ Pfleger /Wiley) or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in literature[2-3]. Isolation of the compounds: The fractionation of the oil was carried over silica gel (230-400 mesh, Loba) by column chromatography using n-hexane (Qualigens) and varying percentages of diethyl ether (Qualigens) in n-hexane as mobile phase[4]. Monitoring was done on pre-coated silica gel TLC plates using iodine as visualizing agent. Repeated column chromatography of the column fractions gave one compound coded as C.

Microorganisms

3-gram negative bacteria viz. Pasteurella multicoda (MTCC 1348), Escherichia coli (MTCC 443), and Salmonella enterica (MTCC 1223), and 2-gram positive bacteria viz. Staphylococcus aureus (MTCC 637) and Bacillus subtilis (MTCC 541) were used for the study of antibacterial activity. Fungi used were Candida albicans (MTCC 854) and Aspergillus flavus (MTCC 771). Standard pure cultures of these bacteria were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India as Microbial Type Culture Collection (MTCC) and maintained in the laboratory by regular sub culturing on to nutrient agar.

Antimicrobial activity

Antibacterial screening of the oil was done by Disc diffusion method as reported in literature, with slight modification[5]. Then minimum inhibitory concentration (MIC) of the essential oil is determined. Bacterial suspension of 0.1 ml (10 times diluted) was added to the previously prepared nutrient agar plate and bacterial strain was thoroughly spread on the surface of agar media, using a bent rod. The sterilized Whatmann filter paper No.1 disc (5mm in diameter) was thoroughly soaked with essential oil (15µL) and placed in the inoculated plates. Gentamycin and Nystatin were used as a reference drugs. Fine pointed forceps were used to place the disc on the previously inoculated plates with the maximum possible aseptic precautions. The discs were firmly pressed against the nutrient agar medium so that they come in complete contact with the agar surface. The discs were placed at equal distances from each other on the seeded plates and the plates were incubated at 370 C overnight, to observe the zone of inhibition around the disc. They were then compared with the zone of inhibition using standard antibiotic after overnight incubation on the nutrient agar plates.

Result and Discussion

Essential Oil Composition The essential oil (yield 0.1%; v/w) obtained from leaves of *Artemisia annua* s was analyzed by using GC-FID and GC-MS.

A total of 24 constituents, representing 74.47% of the total oil, have been elution from an HP-5 column. Essential oil showed the dominant presence of sesquiterpene hydrocarbons (56.86%) followed by oxygenated sesquiterpenes (15.58%) and oxygenated monoterpenes (13.08%). The major constituents of sesquiterpene hydrocarbons were (Z)-β-Farnesene (18.80%), Germacrene D (10.84%) and β-Caryophyllene (3.39%). Oxygenated monoterpenes comprised dihydro citronellol (15.08%) as the representative constituent while α-Cadinol (2.35%), Trans-Arteannuic identified. The composition of the essential oil obtained from leaves of *Artemisia annua* with the retention indices, retention time, percentage composition and identification methods. Compounds are listed in order of their alcohol (3.05%), Zerumbone (2.00%) and Humulene epoxide II (2.85%) were found as major Oxygenated sesqui-terpenes. Aldehyde and hydrocarbon were found in relatively smaller amounts consisting of n-Nonanal (2.79%) and Longicyclene (2.02%) respectively. To the best of our knowledge, this is the first report on the presence of dihydro citronellol (15.08%) in the genus. Earlier, the leaf oil of Senecio chrysanthemoides showed the presence of Germacrene D (10.84%) as main constituent. while in present study, oil was rich in (Z)-β-Farnesene (18.80%) with absence of β-thujone[(6)]. Chemical variation of essential oils has been attributed to difference in environmental and genetic factors[(7)]. Furthermore, ecological factors, particularly, light and temperature have also been reported to influences the production of essential oils as well as other active agents in plants.

Table- Essential oil composition of *Artemisia annua*

Compounds	RT	aLRI	Peak Area (%)	Identification
α-Zingiberene	31.418	1495	1.68	a.b
α-Muurolene	31.648	1499	1.25	a.b
γ-Cadinene	32.116	1513	0.37	a.b
Germacrene D-4-ol	32.279	1574	1.63	a.b
Spathulenol	32.410	1576	1.21	a.b
β-Caryophyllene	28.999	1418	3.39	a.b
(Z)-*β*-Farnesene	30.189	1443	18.80	a.b
γ-Muurolene	30.876	1477	0.69	a.b
Germacrene D	31.091	1480	10.84	a.b
n-Nonanal	8.492	1098	2.79	a.b
Dihydro citronellol	16.876	1196	15.08	a.b
Longicyclene	26.426	1373	2.02	a.b
α-Copaene	27.417	1376	1.40	a.b
β-Maalene	27.686	1380	0.24	a.b
Iso-longifolene	27.919	1387	2.81	a.b
Longifolene	28.005	1402	1.65	a.b
β-Isocomene	28.693	1403	1.74	a.b
Caryophyllene oxide	34.339	1581	1.98	a.b
Humulene epoxide II	35.208	1606	2.85	a.b
Trans-Arteannuic alcohol	36.112	1607	2.05	a.b
epi-*α*-Muurolol	36.466	1641	1.49	a.b
α-Cadinol	36.799	1653	2.35	a.b
Zerumbone	37.130			

Conclusions

The essential oils obtained from the leaves Artemisia *annua* containing (Z)-*β*-farnesene (18.80%), dihydro citronellol (15.08%) and germacrene D (10.84%) as major constituents showed interesting antibacterial and antioxidant activities which make this essential oil a potential industrial resource of new products. Therefore, isolation, characterization and biological activities of major constituents of essential oil will be the further research findings.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

References

1. Gaur, R.D. Flora of the District Garhwal, North West Himalaya. *Transmedia*, 1999.
2. Hammond, G.B.; Fernandez, I.D.; Villegas, L.F. and Vaisberg, A.J. A survey of traditional medicinal plants from the Callejon de Huaylas. *J. Ethnopharmacol.*, 1998, **61**:17-30.
3. Uzun, E.; Sariyar, G.; Adsersen, A.; Karakoc, B.; Otuk, G.; Oktayoglu, E. and Pirildar S. Traditional medicine in Sakarya Province (Turkey) and antimicrobial activities of selected species. *J. Ethnopharmacol*., 2004, 95:287-

296.

4. Adams, R.P. Identification of essential oil components by Gas chromatography Quadrupole Mass spectrometry. *Allured Publ Corp. Carol Stream, IL, U.S.A,* 2004.
5. Adams, R.P. Identification of essential oil components by Gas chromatography/ Mass spectrometry (4th Ed.). *Allured Business Media, Carol Stream. IL, U.S.A,* 2009.
6. Mishra, D.; Joshi, S.; Sah, S.P.; Dev, A. and Bisht, G. Chemical composition and antimicrobial activity of the essential oils of Senecio rufinervis DC. (Asteraceae), *Indian Journal of Natural Product and resources,* 2011, vol 2, (1): 45.
7. Singh, G.; Marimuthu, P.; Murali, H.S. and Bawa, A.S. Antioxidative and antibacterial potential of essential oils and extracts isolated from various spice materials. *J. Food Safety,* 2001, 25:130-135.

Synthesis and bio-evaluation of heterocyclic compound as antimicrobial agents

Neetu Tripathi

*Assistant Professor, Department of Chemistry, M.K.P. P.G. College, Dehradun, Uttarakhand, India

***Email: neetutripathi2622@gmail.com**

DOI 10.51129/ujpah-2024-36-1(7)

Received – 26 December, 2023
Revised – 15 January, 2024
Accepted – 20 January, 2024
Published – 29 June, 2024

Abstract- 1, 4-Dihydropyridines (1, 4-DHPs) are Heterocyclic compounds, an important class of molecules in the field of drugs and pharmaceuticals. Dihydro-pyridine nucleus possess various activities like Vasodilator, bronchodilator, anti-atherosclerotic, antitumor, antidiabetic and hepatoprotective agents. Interest in 1,4-dihydropyridines is due to nicotinamide adenine dinucleotide (NADH), a coenzyme, with unique ability to reduce various functional groups in biological systems. Very recently, dihydropyridines have been reported for resistance reversal properties and antitubercular activities and act as calcium channels blockers. They inhibit the movement of calcium across membrane of myocardial and smooth muscle and their block impulse formation and inhibit conduction velocity and contraction. Further recently such compounds are known to have antibacterial and antifungal activity. Present work is based on the synthesis and bio-evaluation of substituted derivative of 1, 4-Dihydropyridine.

Keywords: 3-nitro Benzaldehyde, Ammonium acetate, antimicrobial activity, Agar nutrient, DMSO

Introduction

Dihydropyridines (DHPs) have very important role in biological activity. The most important biological activity associated with 1,4 Dihydropyridine are calcium channel blocker and there use in treatment of cardiovascular diseases and hypertention.1,2. Calcium channels blockers like 1,4 Di-hydro pyridine, Diphenyl alky amine, Benzothiazepines etc. are developed to inhibit the movement of calcium across membrane of myocardial and smooth muscle and there block impulse formation (automaticity) and inhibit conduction velocity and contraction. As a result of which the Calcium channels blockers have therapeutic utility in angina, arrhythmias, hypertension, antitumer, antidiabetic and other cardio vascular disorder.3,4,5 Calcium channels blockers do not cause fluid retention and can be used equally effective alone for the treatment of mild to moderate hypertension. The efficiency of Calcium channels blockers is enhanced by the concomitant use of β-adrenergic antagonist5, ACEs and methyldopa. Dihydropyridine nucleus is very common in many drugs. DHPs are also known as neuroprotectants, anti-platelet aggregators and are important in Alzheimer's disease

as anti-ischemic agents. They act as Vasodilator, bronchodilator are reported as antioxidants, antitubercular and known to have drug reversal property6-9. These are also known to act as neuroprotectants anti-platelet aggregators. They are also very important in the treatment of Alzheimer's disease as anti-ischemic agents.10. It is interesting to note that an enzyme Nicotinamide Adenine Dinucleotide (NADH) is also related to 1,4 Dihydro-pyridine nucleus. This co-enzyme plays very important role in biological system. NAD+ is an important cofactor used in energy transfer process in all living cells. In plants Dihydropyridine nucleus is mostly found in alkaloids. ref In biological system many metabolic processes involve redox reactions of Nicotinamide adenine dinucleotide (NAD+) reduces its pyridine ring into NADH. The plant Senna spp. is a natural alkaloids of pyridine classes.ref

Several pyridine derivates like pyridinyl thiazyolyazepidinanones, pyridinyl thiazolydiols etc. have been employed for insecticidal, antifungal and antibacterial activity and many compounds have found to exhibit insecticidal and antifungal activity.11-13 The insecticidal and a in vitro antifungal activity was found against different strains of fungi like Aspergilus fumigatus, Candida albicans, etc[14,15]

Review of Literature

Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms, and chemotherapeutic agents, which are chemically synthesized. A hybrid substance is a semi synthetic antibiotic, where in a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of micro-organisms, can be synthesized entirely by chemical means. They might be referred to as synthetic antibiotics to distinguish them from the chemotherapeutic agents. Anti-biotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes. The range of bacteria or other microorganisms that is affected by a certain antibiotic is expressed as its spectrum of action. Antibiotics effective against prokaryotes which kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be broad spectrum. If effective mainly against Gram-positive or Gram-negative bacteria, they are narrow spectrum. If effective against a single organism or disease, they are referred to as limited spectrum. Hence, it was thought to prepare 1,4 Dihydropyridine derivatives for its pharmacological importance.14-18 It was reported that compounds having dihydropyridine nucleus are active molecules to fight against infectious diseases. They have been found to have very potent antibacterial activity against gram-negative bacteria and high antifungal activity against Candida albicans. Such compounds have been investigated for various other bacteria like Staphylococcus epi-dermis and found to have binding interaction with its protein. Hence, the scientific community found the 1,4 Dihydropyridine derivatives a new class of compounds as anti-bacterial and antifungal agents[16-18].

Aim of Study

1,4-dihydropyridine is a six membered aromatic ring containing N at 1 position are very important molecules. The hetero-cyclic ring is the common feature for various pharmacological activities such as Ca^{++} channel blockers, vasodilators and their role as drugs for the treatment of cardiovascular diseases. Some drugs like such as, nifedipine and nitrendipine are used for the treatment of hypertension and angina pectoris. In view of this context, the present study was carried to prepare some new antimicrobial agents as possible inhibitor of bacteria and fungi.

Material and Methods

A synthesis of hybrids of 1,4-dihydropyridines was undertaken in the present study. The compound has also been screened against certain strains of bacteria and fungi.

Synthetic Strategy

Since the first report of the Hantzsch synthesis for 1,4-dihydropyridines a number of strategies have been developed for the synthesis of Dihydropyridines[19-22] In this present study the compound has been synthesized by modified Hantzsch synthesis. To the magnetically stirred slurry of 4 Ao molecular sieve (400mg) in ethylene glycol (20ml), Acetyl Acetone 4.1ml, 39.70 milli mole) and Ammonium Acetate (1.530gms, 19.85 milli mole) was added at room temperature, the reaction mixture was heated up to 60^{o}C and continued for 15 minute and followed by addition of 3-nitrobenzaldehyde (3gms, 19.85 milli mole) and Tetra butyl ammonium bromide (500mg) as a catalyst and stirring was continued till the disappearance of aldehyde. Reaction was monitored by TLC. After completion of reaction final compound was obtained by evaporation of solvent. The reaction mixture was then poured into the cold water and the precipitate obtained was the filtered and dissolved in suitable solvent (chloroform) anhydrous Sodium sulphate (Na_2SO_4) was then added to absorb the moisture and filtered. The solvent chloroform was evaporated under reduced pressure and the product obtained was dried and weighed. The product obtained was purified by column chromatography.

Yield	= 5.146 gms
Solubility	= Chloroform and Ethyl acetate
Physical App.	= Solid
Element Present	= Nitrogen
Molecular Formula	= $C_{17}H_{18}N_2O_4$
Formula Weight	= 314.335

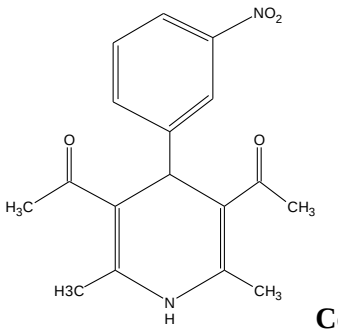
The following reaction shows synthesis of Dihydropyridine based heterocyclic compound.

$C_7H_5NO_3$ + CH_3CONH_4 + $CH_3COCH_2COCH_3$

3-Nitrobenzaldehyde Am. Acetate Acetyl Acetone

Compound [I]

1. 4 A° Molecular Seive
2. Ethylene Glycol
3. TBAB
4. 70^{O}C to 80^{O}C, 3 hrs



Compound I

Bio-evaluation

Synthesized compound was then used for the determination of Antimicrobial activity. 200 mg/ml (in alcohol) dilution was prepared for each strain.

Microorganism Used

Fresh culture of following bacteria & fungi were used in the study:

Bacterial species

1) Bacillus cereus
2) Staphylococcus aureus
3) Proteus mirabilis
4) Escherichia coli

Fungal species

1) Aspergillus niger
2) Aspergillus tereus
3) Penicillium crysogenum
4) Aspergillus japanicus

Culture Media Inoculums

All-purpose Muller Hinton Agar media, (Himedia, No. 173) was used in this study microbial culture, freshly grown at 37oC were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5CFU/ml.

Antimicrobial Assay

The agar well diffusion method was used. 1 ml of diluted inoculums (105FU/ml) of test organism was mixed on the Muller Hinton agar media (for bacteria) and scabbard's agar (for fungus), shacked and pour in sterilized Petri plates. The wells of 8mm diameter were punched into the agar medium. To each well 200mg/ml of compound was added and allowed to diffuse. The compound was tested against each organism in triplicate set. The plate were then incubated aerobically 37oC for the bacteria stains for 24 hrs and at 27oC for the fungus for 72 hrs.

The antimicrobial activities then tested for compound and recorded as the diameter of the resulting growth inhibition in millimeters.

In the present work 1,4 dihydro-pyridine derivative has been synthesised by an efficient method. One mole of 3-Nitrobenzaldehyde 99% on reaction with 2 mole of β-keto compound and 1 mole of ammonium acetate in the presence of ecofriendly solvent ethylene glycol underwent the condensation reaction to give N, N'-disubstituted 1,4-dihydropyridine compound [1] in quantitative yield. In above method 4 A˚ molecular sieves play very important role to carry out the reaction in forward direction by simultaneous absorption of water released during the condensation reaction. TBAB used as phase transfer catalyst to increase nucleophillicity of attacking nucleophile in reaction mechanism. Compound [I] obtained by above mentioned method was characterized with the help of nitrogen element detection and I.R. spectroscopy. The synthesis of compound [I] is evaluated for antimicrobial activity. Since pyridine and its dihydropyridine derivatives are well known to show antibacterial activity. This prompted us to investigated the synthesized compound as possible inhibitor of pathogenic microorganisms.

The compound [I] was screened against two gram positive i.e. Staphylococcus aureus and Bacillus cereus and two gram negative bacterial strains i.e. Escherichia coli and Proteus mirabilis. The compound

[I] was dissolved in DMSO (Dimethyl Sulfoxide) an concentration of 200 mg/ml was made. The activity result of compound [I] against above mentioned strains has been given in table (1).

Table-1 The antibacterial activity of compound I

S. No	Bacterial Species	Kind of Bacteria	Concentration	Inhibition zone in (mm)
1	Bacillus cereus	Gram positive	200mg/ml	26mm
2	Staphylococcus aureus	Gram positive	200mg/ml	29mm
3	Proteus mirabilis	Gram negative	200mg/ml	-
4	Escherichia Coli	Gram negative	200mg/ml	26mm

Results and Discussions

The above activity result shown is very encouraging. Compound [I] was found to have inhibitory activity against both gram positive as well as gram negative bacteria however the synthesized compound was not active against all test organism. Inhibition zone shown by compound (1) are 26 mm,29 mm and 26 mm against Bacillus cereus, Staphylococcus aureus and Escherichia coli respectively.

The antibacterial activity result of compound [I] against the four different strains of bacteria is also shown in shown in pictures 1, 2, 3 and 4.

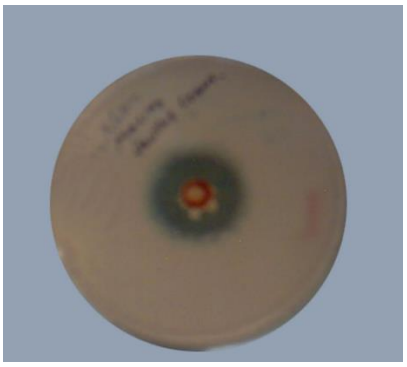


Figure – 1 Bacillus cereus

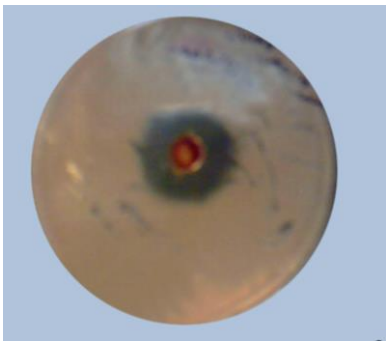


Figure -2 Staphylococcus aureus

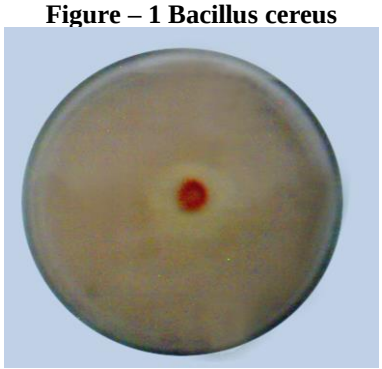


Figure 3 Proteus mirabilis

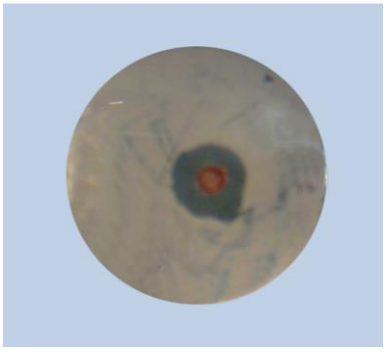


Figure 4 Escherichia coli

Further the synthesized compound [I] was also evaluated for antifungal activity. The fungal strains used as organisms are Aspergillus niger, Aspergillus tereus, Aspergillus japanicus and Penicillium crysogerium. The compound was dissolved in DMSO (Dimethyl sulfoxide) and 200mg/ml concentration was made and inoculated to the test organism, after 72 hours a portion of fungal colony was killed by the test sample and this was appeared as cleaned zone around the test compound [I]. This zone was measured in mm scale and the result obtained are a 16.33 mm (Aspergillus nijer), 14 mm (Aspergil-lustereus), 21mm (Aspergillus japanicus) and 23 mm (Penicillium crysogerium) as given in Table (2).

Table 2: The antifungal activity of compound [1]

S. No.	Test organism	Concentration of compound [I]	Zone of inhibition in (mm)
1.	*Aspergillus nijer*	200mg/ml	16.33mm
2.	*Aspergillus tereus*	200mg/ml	14 mm
3.	*Penicillium crysogerium*	200mg/ml	23mm
4.	*Aspergillus japanicus*	200mg/ml	21mm

Conclusion

Conclusion: The synthesized compound [1] i.e.(3,5-Diacetyl-2,6-dimethyl-4-(3-nitro phenyl-1,4-Dihydropyridine) was synthesized in quantitative yield and it was evaluated for antibacterial and antifungal activity. The maximum inhibition was noticed against the bacterium Staphylococcus aureus and better antifungal activity was observed against Penicillium chrysogenum.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Bossert, F.; Meyer, H. and Wehinger, E. *Angew Chem. Int. Ed. Engel*, 1981, 20:762-769.
2. Nakagama, H. and Asoka, Y. *Heterocyclic*, 1996, 42:901-909.
3. Sauchines, A. and Duburs, G. *Heterocycles*, 1988, 27:269-289.
4. Mager, P.P.; Coburn, R. A.; Solo, A. J.; Triggle, D. J. and Rothe, H. *Drug Des. Discov*., 1992,8,273-289.
5. Manhold, R.; Jablonba, B.; Viogdt, W.; Schoenfinger, K. F. and Schravan, K. *Eur. J. med. Chem.*, 1992, 27:229-235.
6. Shridhar, R. and Perumal, P. *Tetrahedron*, 2005, 61:2465.
7. Trivedi, A. R.; Dodia, D. K.; Dholaria, B. H.; Kataria, V. B.; Bhuva, V. R. and Shah, V. H. *Biorgan. Med. Chem. Lett*., 2011, 21:5181–5183

8. Boer, R. and Gekker, V. *Drugs future*,1995, 9:509.
9. Rao, S. N. and Laxmi, K. L. *Sci. Rev. Chem. Comm.*, 2013, 3:141–149
10. a) Bretzel, R. G.; Bollen, C. C.; Maeser, E.; Fedrlin, K. F. Am. *J. Kidney Dis*., 1993, 21:53.

    b) Lim, Shey-Li; Voon, Chia Pao; Guan, Xiaoqian; Yang, Yi; Gardeström, Per; and Leong Li, Boon. In planta study of photosynthesis and photorespiration using NADPH and NADH/NAD+ fluorescent protein sensors; *Nature Communications*, 2020, volume 11, Article number: 3238

    c) Bidisha Ukil, Nikhilesh Joardar, Santi Prasad Sinha Babu, and Larisha M. Lyndemcorresponding ; Effect of Senna plant on the mitochondrial activity of Hymenolepis diminuta, J Parasit Dis. 2022 Mar; 46(1): 139–151.

11. Manvar, A.T.; Raghuvir, R.S. and Pissurlenkar. *Mol. Divers.*, 2010,14:285–305
12. Olejnikova, P.; Svorc, L.; Olsovska, D.; Panakova, A.; Vihonska, Z.; Kovaryova, K. and Marchalin, K. C. *Yednik Am. Pharma*, 2014, 33:49(1993).
13. Elbert, A.; Becker, B.; Hartwig, J. & Erdelen, C. *Pflanzenschutz Nachrichten,* 1991, 44:113.
14. Kennedy, A. D. & Summer, A. L. *J. Heterocycl. Chem.*, 1981, 18:409.
15. Malani, Alimamad; Makwana, Atul; Monapara, Jahnvi; Ahmad, Iqrar; Patel, Harun and Desa, Nisheeth. *Journal of Biochemical and Molecular Toxicology*, 2021, Vol. 35, Issue 11.
16. Dharambeer, S.; Malhi, Harvinder; Sohal, S.; Singh, Kishanpal; Almarhoon, Zainab M.; Bacha, Abir Ben and Maha I. *Al-Zaben, ACS Omega*, 2022, 7:18:16055-16062.
17. Sircar, I. 1, 4-Dihydropyridines useful in the treatment of cardiovascular disorders. *Eur. Pat. Appl.*, 1987, 106:206747-176173.
18. Shah, A.; Vora, V.; Desai, B. and Gaveriya, H. *Heterocycl. Commun.* 2001, 7:481.
19. Dondoni, A.; Massi, A. and Minghini, E. *Synlet*, 2002, $p_{p.}$89.
20. Zolfigol MA, Safaiee M. Synlett; 2004. $p_{p.}$ 827.
21. Tewari, Neetu; Dwivedi, Namrta; Tripathi, R.P. Tetrabutyl ammonium hydrogen sulphate catalyzed eco-friendly and efficient synthesis of glycosyl 1,4-dihydropyridine. *Tetra-hedron Lett.*, 2004, 45(49):9011-9.

Bactericidal action of Tulsi (*Ocimum sanctum*) leaf extract against four human pathogenic bacteria

***Mirza Azim Beg, Ragib Ali and Rahisuddin**
Himalaya Wellness Company, Faridabad Unit, Haryana, India
*Email: azim_0088@yahoo.com

DOI 10.51129/ujpah-2024-36-1(8)

Received – 12 June, 2024
Revised – 13 June, 2024
Accepted – 15 June, 2024
Published – 29 June, 2024

Abstract- Bacterial ailment remains a serious health problem due to antibiotics resistance. Tulsi is an aromatic medicinal herb belongs to Lamiaceae family. Therapeutic use of Tulsi is as old as 4000-5000 B.C. Carvacrol, a mono-terpenic phenol present in Tulsi leaves has emerged for its wide spectrum activity extended to food spoilage or pathogenic fungi, yeast and bacteria as well as human, animal and plant pathogenic microorganisms including drug-resistant and biofilm membrane forming microorganisms. Therefore, the present study performed to evaluate *in-vitro* bactericidal activity of different extracts (Aqueous, Methanol, Ethanol and Chloroform) of *Ocimum sanctum* leaves against four human pathogens, *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella Spe.* with the reference of Ampicillin and tetracycline antibiotics.

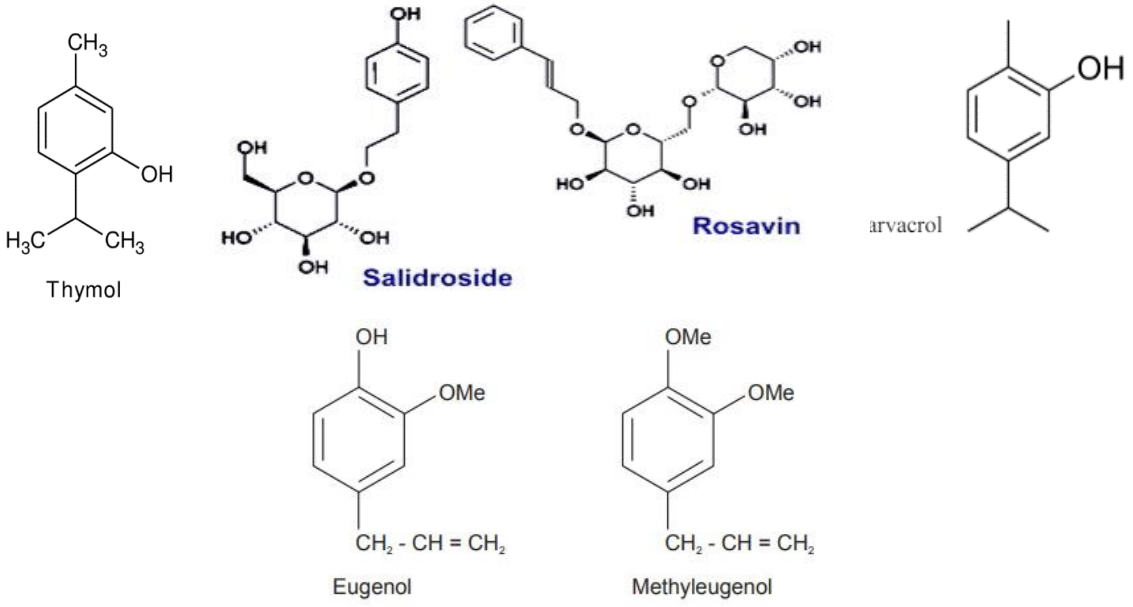
Keywords: Antibacterial activity, Tulsi (*Ocimum sanctum*) leaves, Pathogen, Antibiotics, solvent extract.

Introduction

Tulsi (Holy Basil) is an aromatic medicinal herb belongs to Lamiaceae family. It is originated in north central India, however now grows native throughout the eastern world tropics[1]. In Ayurveda, tulsi is considered as "The Incomparable One," "Mother Medicine of Nature" and "The Queen of Herbs," and is known as "elixir of life" due to its both medicinal as well as spiritual properties[2]. According to Hindu 'Padma Puran', Tulsi is a Sanskrit word which means matchless[3] one, considered as a sacred plant, can be found around Hindu shrines. Therapeutic use of Tulsi is as old as 4000-5000 BC[4]. Tulsi leaves is used in a range of conditions including anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, indigestion, hiccups, vomiting, gastric, cardiac and genitourinary disorders back pain, skin diseases, ringworm, insect, snake and scorpion bites and malaria etc[2,5-7]. Tulsi leaves essential oil contains anti-oxidant component, Eugenol (4-allyl-2-methoxyphenol) which can slow progression and improve survival rate in animals with certain types of cancer while further in-vivo study required for its confirmation. Tulsi antioxidant protect against the toxic effects of industrial chemicals such as butyl paraben, carbon tetrachloride, copper sulfate and ethanol, Pesticides such as rogor, chlorpyrifos, endosulfan and

lindane and pharmaceutical products like acetaminophen, meloxicam, paracet-amol, haloperidol and anti-tubercular drugs. Eugenol helps to protect the heart by keeping blood pressure under control and lowering cholesterol levels. Chewing a few leaves of Tulsi on an empty stomach everyday can prevent and protect from heart ailments[8-20]. Eugenol, Methyl Eugenol and Caryophyllene present in Tulsi leaves collectively help to restore the function of pancreatic beta cells (cells that store and release insulin) by increase insulin sensitivity, lowering blood sugar and hence treating diabetes effectively[21-22]. Carvacrol, a monoterpenic phenol present in Tulsi leaves has emerged for its wide spectrum activity extended to food spoilage or pathogenic fungi, yeast and bacteria as well as human, animal and plant pathogenic microorganisms including drug-resistant and biofilm membrane forming microorganisms. Many research articles and the recent patents claimed in order to highlight its future applications as a new antimicrobial agent. It could concern either the natural preservation in the cosmetic and food industries or an alternative which supports the conventional antimicrobial protocols. However, more investigation and in vivo studies must be carried out before using this molecule in the future. The mice fed with Carvacrol along with high-fat diet had been observed significantly lowered cholesterol value at the end of the 10 weeks. Thus cholesterol-lowering effect of tulsi oil is thought to be the result of the phenols carvacrol and thymol present in it[23-26]. Tulsi contains Rosavin which is the primary alcohol glycoside responsible for antidepressant actions[27]. Salidroside is another primary anti-depressant and anxiolytic compound found in Tulsi. It has been used for centuries; especially within traditional Chinese medicine as a work enhancer. Currently, many body-builders and endurance athletes consume it as a supplement[27-28].

Structure of important chemical constituents of Tulsi plant (Source: Google web)



Material and Methods

Plant sample Collection- Tulsi (*Ocimum sanctum*) leaves was collected from the store of Himalaya Wellness Company, Faridabad (Haryana) and thoroughly washed in running tap water and air dried at room temperature in the shade for 5 days, then powdered using a mixer grinder and stored in an air tight container at 4 ± 2 ℃ for further use.

Organic solvent extraction- Four different solvents (methanol, ethanol, chloroform and distilled water) were used to prepare leaf extract. Twenty gram of Tulsi (*Ocimum sanctum*) powder was mixed with 200 mL of each solvent separately and kept in an orbital shaker at 120 rpm for 48 h at room temperature. Then the extract was filtered using a Whatman No. 1 filter paper or centrifuged at 5000 rpm for 10 min and transferred to a petri-dish and allowed to evaporate and weighed. Finally, all dried extract were dissolved in 50 mg/mL concentration of respective solvents.

Antibacterial activity- The different solvent extracts were subjected to antibacterial assay by Kirby Bauer disc diffusion method using Muller Hinton agar plates against four human bacterial pathogens of *Escherichia coli*, *Salmonella spe. , Pseudomonas aeruginosa* and *Streptococcus aureus*. The pathogens were obtained from Department of Microbiology Himalaya Wellness Company Faridabad Haryana and maintained in Lysogeny broth (LB) broth at 37 ± 2 ℃. The extract discs were prepared using sterile Whatmann No.1 filter paper (6 mm diameter) by soaking in the extract (5 min) and air dried. For positive control 10 µg penicillin and 50 µg tetracycline disc was used. The 50 µL of 12 hours old cultures was speeded on Muller Hinton agar plates and placed the extract disc and positive control disc and incubated for 24 hours at 37±2 ℃, and measure the zone of inhibition (ZoI) as in diameter.

Results and Discussions

Ethnobotanical investigations may offer important clues for the identification and development of traditional use of medicinal plants in a modern medicines[29]. Nowadays antibiotic resistance to bacteria is a major problem throughout the world[30]. From the past 20 years, scientist from virtually every corner of the world have documented that increasing proportions of bacteria are resistant to penicillin and other important antibiotics. It is necessary to find the natural antibiotics[29, 31] to restrain resistance and our studies have shown that organic solvent extract of *Ocimum sanctum* leaves can act as potential antibacterial agents that may be useful in the pharmaceutical industries.

The Table-1 shows the zone of inhibition of Tulsi (*Ocimum sanctum*) leaves extract against four human pathogenic bacteria. The ZoI at maximum of 24 mm was observed in methanol extract of Tulsi (*Ocimum sanctum*) against *S. aurus*, which is a Gram positive bacterium. While distilled water, ethanol and chloroform extract observed maximum of 22 mm, 21 mm and 19 mm ZoI respectively against same *S. aurus*. Therefore, we can say that Tulsi leaves extract have highest bactericidal activity against Gram positive pathogens, however it also shows good bactericidal effect against Gram negative bacteria of

E. coli which was at maximum of 20 mm ZoI in Methanol extract, 18 mm in distilled water extract, 16 mm in Ethanol extract and 14 mm in Chloroform extract. The positive antibiotic control of tetracycline and penicillin expressed the bactericidal against all four pathogens used, however, the Tulsi leaves methanolic extract was reached maximum of 24 mm ZoI which is higher than the ampicillin (10 µg) shows 7 mm ZoI and Tetracycline (50 µg) shows 15 mm ZoI against the same pathogen of *S. aureus.*

Table-1 Zone of inhibition (diameter in mm) of different of *Ocimum sanctum* leaves against four human pathogens

Test substances	Zone of inhibition diameter (mm)			
	E. coli	***Salmonella Spe.***	***P. aeruginosa***	***S.aureus***
Methanol extract	20	18	21	24
Ethanol extract	16	17	19	21
Chloroform extract	14	15	17	19
Distilled water extract	18	16	20	22
Ampicillin (10 µg)	7.5	15	12.5	7
Tetracycline (50 µg)	16	14	12.5	15

The present study prove that methanol, distilled water, ethanol, and chloroform extract of *Ocimum sanctum* leaves could be able to inhibit four tested bacteria. Currently due to the emergence in antibiotic resistant infections, the search for new natural alternative to treat infections is entirely necessary and in this regard *Ocimum sanctum* leaves extract can give an alternative source for design of novel medicines.

Conclusion

This study evidently concludes that the Tulsi (*Ocimum sanctum)* leaves extract has ample potential to inhibit four common human pathogenic bacteria at unique intensity, however, the methanol extract was the best among other solvents (Water, Ethanol and Chloroform).

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Yadav, Kishor Raj. “Medicinal chemistry of Tulsi” (Ocimum sanctum - holy basil). *J.E.T.I.R.*, February 2019, Volume 6, Issue 2, www.jetir.org (ISSN-2349-5162).
2. Singh, N.; Hoette, Y. and Miller, R. Tulsi: The Mother Medicine of Nature. *Lucknow: International Institute of Herbal Medicine*, 2nd ed.
3. Labh Kayastha, Babita. *Journal of medicinal plant studies*, 2004, Vol-2, ISSN- 2320:3862.
4. Rahnamaeian, M. Proc. R. Soc., B282, 20015:0293.

5. Mohan, L.; Amberkar, M. V. and Kumari, M. *Ocimum sanctum linn*. (TULSI)-an overview. *Int. J. Pharm. Sci. Rev. Res*., 2011, 7:51–3.
6. Pattanayak, P.; Behera, P.; Das, D. and Panda, S. K. *Ocimum sanctum Linn.* A reservoir plant for therapeutic applications: An overview. *Pharmacogn Rev*. 2010, 4:95–105.
7. Mondal, S.; Mirdha, B. R. and Mahapatra, S. C. The science behind sacredness of Tulsi *(Ocimum sanctum Linn.) Indian J. Physiol. Pharmacol*. 2009, 53:291-306.
8. Shah, K. and Verma, R. J. Protection against butyl p-hydroxybenzoic acid induced oxidative stress by *Ocimum sanctum* extract in mice liver. *Acta. Pol. Pharm.*, 2012, 69:865-70.
9. Enayatallah, S.A.; Shah, S.N. and Bodhankar, S. L. A study of hepatoprotective activity of *Ocimum sanctum* (Krishna tulas) extracts in chemically induced liver damage in albino mice. *J. Ecophysiol. Occup. Health.*, 2004, 4:89-96.
10. Shyamala, A. C.;; and Devaki, T. Studies on peroxidation in rats ingesting copper sulphate and effect of subsequent treatment with *Ocimum sanctum. J. Clin. Biochem. Nutr.*, 1996, 20:113-9.
11. Bawankule, D.U.; Pal, A.; Gupta, S.; Yadav, S.; Misra, A. and Rastogi, S. et al. Protective effect of Ocimum sanctum on ethanol-induced oxidative stress in Swiss Albino Mice brain. *Toxicol. Int*., 2008, 5:121-5.
12. Verma, P.; Kedia, D.K. and Nath, A. Protective effect of *Ocimum sanctum* leaf extracts against rogor induced ovarian toxicity in *Clarias batrachus Linn. J. Ecophysiology Occup. Health*, 2007, 7:177-84.
13. Khanna, A.; Shukla, P. and Tabassum, S. Role of *Ocimum sanctum* as a geno-protective agent on chlorpyrifos-induced genotoxicity. *Toxicol. Int.*, 2011, 18:9-13.
14. Bharath, B. K.; Anjaneyulu, Y. and Srilatha, C. Imuuno-modulatory effect of *Ocimum sanctum* against endosulfan induced immunotoxicity. *Vet World*, 2011, 4:25-7.
15. Mediratta, P. K.; Tanwar, K.; Reeta, K. H.; Mathur, R.; Banerjee, B.D. and Singh, S. et al. Attenuation of the effect of lindane on immune responses and oxidative stress by *Ocimum sanctum* seed oil (OSSO) in rats. *Indian J. Physiol. Pharmacol*., 2008, 52:171.
16. Makwana, M. and Rathore, H. S. Prevention of hepatorenal toxicity of acetaminophen with *Ocimum sanctum* in mice. *Int. J. Pharm. Technol*., 2011, 3:1385-96.

17. Mahaprabhu, R.; Bhandarkar, A.G.; Jangir, B.L.; Rahangadale, S. P.; Kurkure, N.V. Ameliorative effect of *Ocimum Sanctum* on meloxicam induced toxicity in wistar rats. Toxicol. Int., 2011, 18:130-6.
18. Lahon, K. and Das, S. Hepatoprotective activity of *Ocimum sanctum* alcoholic leaf extract against paracetamol-induced liver damage in Albino rats. *Pharmacognosy Res*., 2011, 3:13-8.
19. Pemminati, S.; Nair, V.; Dorababu, P.; Gopalakrishna, H. N. and Pai, M. R. Effect of ethanolic leaf extract of *Ocimum sanctum* on haloperidol-induced catalepsy in albino mice. *Indian J. Pharmacol*., 2007, 39:87-9.
20. Ubaid, R.S.; Anantrao, K.M.; Jaju, J.B. and Mateenuddin, M. Effect of *Ocimum sanctum* (OS) leaf extract on hepatotoxicity induced by anti-tubercular drugs in rats. *Indian J. Physiol. Pharmacol.*, 2003, 47:465-70.
21. Bokyung, Sung. and Bharat, B. *Advances in Botanical Research*, 2012.
22. Kiferle, C.; Lucchesini, Mariella; Mensuali-Sodi, Anna; Maggini, Rita; Raffaelli, Andrea; Pardossi, Alberto. *Central European Journal of Biology*, December 2011, Volume 6, Issue 6, pp. 946–957.
23. Mohammadi, Z. *Open Access Journal of Chemistry*, Volume 1, Issue 1, 2017, pp. 53-62.
24. Mishra, Punam. Study of antibacterial activity of *Ocimum sanctum*. *American journal of Food technology*, 2011, 6(4):336-341.
25. Piglets, H. K.; Wei, H. X.; Xue, Z.; Zhou, X. and Peng, J. https://doi.org/10.1017/S1751731116001397.Published online: July 15, 2016.
26. Suzuki, T.; Yoshinaga, N. and Tanabe, S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. *Journal of Biological Chemistry*, 2011, 286: 31263– 31271.
27. Bast, F.; Rani, P. and Meena, D. Chloroplast DNA phylogeographic of holy basil (Ocimum tenuiflorum) in Indian subcontinent. *Scientific World Journal*, 2014, 847–482.
28. World Health Organisation. Preventing Chronic Diseases: A Vital Investment: WHO Global Report. Geneva: World Health Organization. Department of Chronic Diseases and Health Promotion, 2005, pp. 18.
29. Kapil, A. The challenge of antibiotic resistance: need to contemplate. *Indian Journal of Medical Research*, 2005, 121(2):83-91.

30. Kaplan, S. L. and Mason, E. O. Management of infections due to antibiotic-resistant *Streptococcus pneumoniae*. *Clinical microbiology reviews*, 1998, 11(4):628-44.
31. Firomsa, Workitu; Tamirat, Abenezer; Tekle, Biniyam; Tesfaye, Biniyam; Shafi, Habib and Nainar, Arumuga. Bactericidal action of Croton macrostachyus leaf extract against common human pathogenic bacteria. *Journal of Medicinal Plants Studies*, 2018, 6(6): 33-36.

Anantamool (*Hemidesmus indicus*) as herbal blood purifier in Ayurvedic medication

[*]S. Farooq, Zafar Mehmood and A. K. Dixit
Himalaya Wellness Company Dehradun
[*]Email: dr.sfarooq.him@gmail.com

DOI 10.51129/ujpah-2024-36-1(9)

Received – 14 June, 2024
Revised – 15 June, 2024
Accepted – 18 June, 2024
Published – 29 June, 2024

Abstract- There are no synthetic medications used for blood purifiers that are effective in purifying the blood and treating various illnesses. Plants have long been a reliable source of medicine. Plants are mentioned in Ayurveda and other Indian literature as being used to treat a variety of human diseases. Herbs referenced in ancient texts or historically used for various disorders have yielded astonishing benefits. Various market formulations are available that aid in blood purification. In this research, we look at a variety of herbal plants that are good for blood purification and cleaning and are commonly found in various market formulations. Anantamool *Hemidesmus indicus* has a lengthy history of use as a blood purifier, tonic, and alterative. In the Ayurvedic medicine system, *Hemidesmus indicus* is a well-known drug. *Hemidesmus indicus* root bark extract possesses antioxidant effects. One of the ways by which this medicine is useful in numerous free radical mediated illness situations is its ability to scavenge free radicals. Furthermore, it has anti-inflammatory properties. Herbal blood purifiers (Raktasaaf) have no single therapeutics activity but have multiple therapeutics activities. The present investigation is designed to test its antimicrobial activity that can validate its use as a blood purifier in different Ayurvedic Formulations. The results revealed promising antibacterial activity against pathogenic bacteria.

Keywords: *Hemidesmus indicus*; Blood purifier; Antioxidant; Anti-inflammatory activity; Ayurvedic medication, antibacterial activity, antimicrobial activity

Introduction

There are no synthetic medications used for blood purifiers that are effective in purifying the blood and treating various illnesses caused by blood pollutants. There are several medications for different problems associated to blood impurities. When these treatments are administered to a person, they have an effect on the condition, but they only cure one disease, and they also have adverse effects. We can use herbal medications to help cure these problems with less adverse effects in herbal medications. We employ Poly herbal formulation in herbal medications. At first, a blood purifier can aid with increased bowel movement, which is a transient phase. It takes two or three days, and it is highly suggested because

it aids in the blood cleansing process. Either starts with tiny doses and gradually increases after two or three days, or starts with the suggested amount and wait for the bowel movement to clear. It stimulates the liver and kidneys, making them healthier and more active. Our skin becomes more healthy, glossy, smooth, and silky with a natural shine as our system is cleansed. Skin that is healthy is less susceptible to diseases and infections[1].

Plants have long been a reliable source of medicine. Plants are mentioned in Ayurveda and other Indian literature as being used to treat a variety of human diseases. When blood becomes polluted owing to poor circulation, it causes ailments such as acne, pustules, and rashes, as well as complications from allergies, a weakened immune system, headaches, jaundice, wrinkles on the face, spinning of the head, hair loss, failing eyesight, and joint tightness.[1]

Anantamool (*Hemidesmus indicus*) family: Asclepiadaceae The plant has a long history of use as a blood purifier, tonic, and alterative. In the Ayurvedic medicine system, *Hemidesmus indicus* is a well-known drug[2]. *Hemidesmus indicus* root bark extract possesses antioxidant effects. One of the ways by which this medicine is useful in numerous free radical mediated illness situations is its ability to scavenge free radicals. Furthermore, it has anti-inflammatory properties.[3,7 and 8]

Herbal blood purifiers (Raktasaaf) have no single therapeutics activity but have multiple therapeutics activity due to poly herbal formulation with lesser side effects. There are different herbal drugs formulations in market which have similar activity of blood purifier and Raktasaaf contain common plants. 'Raktasaaf formulations' not shows only blood purifier property but also have other properties like antibacterial, antifungal, immunomodulators etc. Raktasaaf have greater therapeutics effect then their side effects.

One possible approach is to test plants extracts for their potential to be used against multi-resistant bacteria. India has one of the world's richest flora with about 120 families of plants comprising 1,30,000 species and about 119 secondary plant metabolites are used globally as drugs. The WHO reported that 80% of world population rely chiefly on traditional medicines/herbs for primary healthcare have steadily increased worldwide in the recent years[6]. Keeping in view this study is designed to evaluate the antimicrobial activity of ***Hemidesmus indicus*** .

Material and Methods

Collection of plant materials- *Hemidesmus indicus* roots were collected from the Himalaya Wellness Company Dehradun India. The collected plant material was identified by Dr. Mayaram Uniyal department of Pharmacognosy, Himalaya Wellness Company Dehradun. Roots were washed under the running tap water 2-3 times and once with sterile distilled water and dried under shade and then homogenized to fine powder and stored in air tight container till further use.

Preparation of solvent root extraction- The method of Alade and Irobi[4], (1993) was adopted for preparation of plant

extracts with little modifications. The dried 25 g powdered root soaked separately in 100 ml Hexane, methanol, and aqueous .Each solvents were kept in separate flasks with powdered sample were kept in a rotating shaker for 3 days. The extracts were filtered through whatman Filter paper No.1 and the extracts were reduced to half of its original volume. The organic solvents were concentrated in vacuum using rotary evaporator, while aqueous extract was dried using water bath.

Culture media-The media used for antibacterial test was soyabean casein digest agar/broth of Hi Media Pvt. Ltd. Bombay, India.

Inoculum-The bacteria were inoculated into soyabean casein digest agar /broth and inoculated and incubated at 37 °C for 4 h and the suspension was checked to provide approximately 10^5 CFU/ml.

Microorganisms- The antibacterial activity of the extract was tested individually on G+ve and G-ve bacterial strains. All bacterial strains were obtained from IMTECH, Chandigarh India. The G+ve strain used was *Staphylococcus aureus MTCC 737 and* G-ve bacterial strains were *E.coli MTCC 1687;Psuedomonas aeruginosa MTCC 1688* and *Salmonella enteric MTCC 3858.and Candida albicans MTCC 3017.*

Determination of antibacterial/ anti-candidal activity- The agar well diffusion method (Perez et al; 1990)[5] was modified. Soyabean casein digest agar (SCDA) was used for bacterial cultures. The culture medium is inoculated with the microorganisms suspended in soyabean casein digest broth. A total of 8mm diameter wells were punched into agar and filled with plant extracts and solvent blanks (distilled water, hexane and methanol as the case may be). Standard antibiotic was simultaneously used as positive control. The plates were then incubated at 37°C for 18 h. The antibacterial/anticandidal activity was evaluated by measuring the inhibition zone diameter observed.

Wells were filled with 0.1 ml of 20 mg/ml concentration of each sample (2mg/well). Bioactivity was by measuring Diameter of Inhibition Zones (DIZ) in mm.

Results and Discussion

Among all the tested extracts hexane extract was found to have maximum zone of 22mm against *Staphylococcus aureus* (Table-1, Figure-1 and 2 & Plate-1) followed by *E.coli* (18mm),*Candida albicans* (18mm), *Pseudomonas aeruginosa* (16mm) and *Salmonella enterica* (15mm).The significant antimicrobial effect of *Hemidesmus indicus* against all the pathogen confirmed that the compound present in the crude extract are responsible for the effective antimicrobial activity.

The traditional therapeutic indications of *Hemidesmus indicus as* blood purifier studied appear to have a fairly good degree of correlation with their antimicrobial activity. The herb *Hemidesmus indicus* appear to have broad spectrum of action, it could be useful in antiseptic, disinfectant formulations and in chemotherapy. The antibacterial activities of the herb is particularly noteworthy, considering the

importance of these organisms in infection control and as blood purifier.

Table-1 Antimicrobial activity of *Hemidesmus indicus* **root extract**

S. No.	**Test microorganisms**	**Diameter of zone of inhibition(mm)**			
		Hexane extract	Methanol extract	Aqueous extract	**+VE Control Ciprofloxacin 30µg/ml**
1.0.	*Staphylococcus aureus MTCC 737*	22	16	NAD	25
2.0.	*E.coli MTCC 1687*	18	14	NAD	21
3.0.	*Pseudomonas aeruginosa MTCC 1688*	16	12	NAD	22
4.0.	*Salmonella enterica MTCC 3858*	15	13	NAD	21
5.0.	*Candida albicans MTCC 3017*	18	16	NAD	----

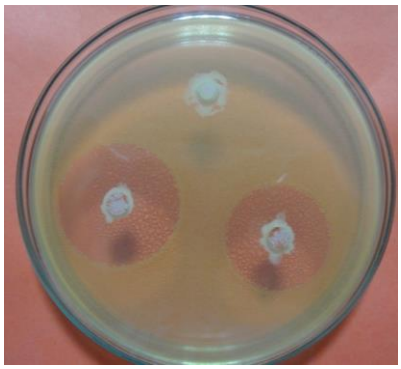


Plate-1 Antibacterial activity of Hexane extract against *Staphaureus* **(MTCC 737)**

.

Figure-1 Antimicrobial efficacy of Hexane and Methanol extract of *Hemidesmus indicus* **in the form of Diameter of zone of inhibition**

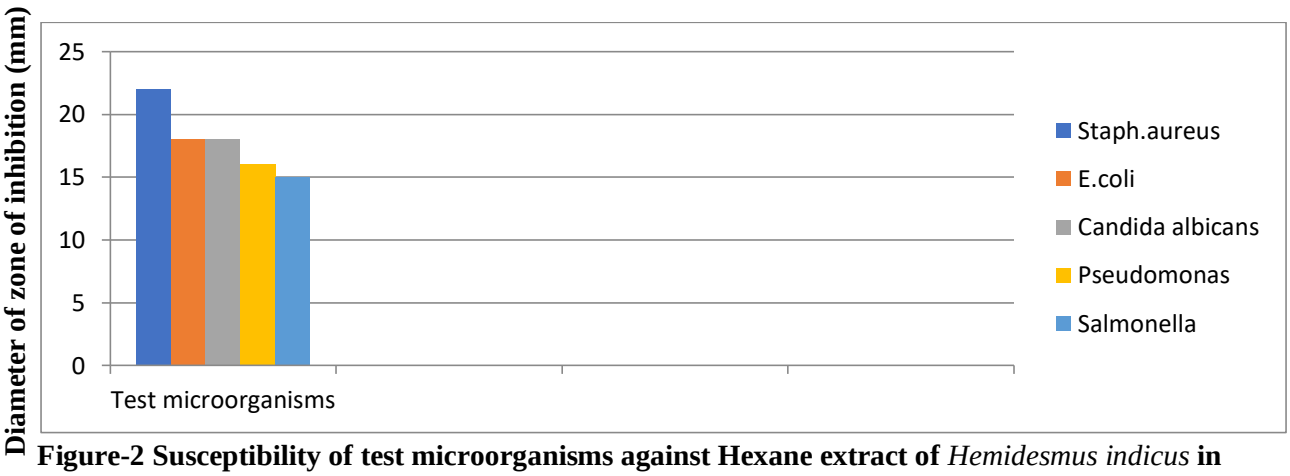


Figure-2 Susceptibility of test microorganisms against Hexane extract of *Hemidesmus indicus* in the form of diameter of zone of inhibition(mm).

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Sagar Ayurvedic Pharmacy India: Association of herbal formulations. Available from: http://www. satveda.com/home.php, 1932.

2. Anoop, A. A Review on Indian Sarsaparilla, Hemidesmus indicus (L.) R. Br. *J. Biol. Sci.*, 2008, 8(1):1–12.

3. Anita, M.; Purnima, A. and Varadharajan, M. Antioxidant activity of leaf of *Hemidesmus indicus* L R Br var pubescens W A Hkf Periplocaceae- an in vivo analysis. *Spatula*, 2011, 1(2):91–100. doi:10.5455/spatula.20110524114 752.

4. Alade, P. I. and Irobi, O. N. Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiana*. *Journal of Ethnopharmacology*, 1993, 39:171-174.

5. Perez, Cristina and Anesini, Claudia. In vitro antimicrobial activity of Argentine folk medicinal plants against *Salmonella typhi*. *Journal of Ethnopharmacology*, 1990, 44:41-46.

6.World Health Organization (WHO) Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram.*, 1993, 28:13-14.

7. Nadkarni, A.N. *Indian Materia Medica.*, 1989, Vol. I, Popular Book Depot, Bombay, India

8.Chopra, R. N.; Nayar, S. L. and Chopra, I. C. *Glossary of Indian Medicinal Plants*, CSIR, New Delhi, India, 1956.

Inhibitory activity of *Alpinia galanga* (Kulanjan) against pathogenic bacteria

Medha Panwar and Vikas Singh Mishrwan
Department of Chemistry, Uttaranchal University, Dehradun, India
*Email:medhapanwar811@gmail.com
DOI 10.51129/ujpah-2024-36-1(10)

Received – 14 June, 2024
Revised – 15 June, 2024
Accepted – 18 June, 2024
Published – 29 June, 2024

Abstract-Infectious disease are the world's leading cause of premature deaths, killing almost 50 000 people every day. With the continuous use of antibiotics microbes have become resistant. This has created immense clinical problems in the treatment of infectious diseases.

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Plant materials remain an important recourse to combat serious diseases in the world. They are used directly as therapeutic agents, as well as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. The search for potent antibacterial agents has now been shifted to plants. One approach is to screen local medicinal plants for possible antimicrobial properties.

According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Yet a scientific study of plants to determine their antimicrobial active compounds is a comparatively new field. There are many published reports on the effectiveness of traditional herbs against pathogenic bacteria, viruses and fungi and as a result plants are still recognized as the bedrock for modern medicine to treat infectious diseases. Keeping in view of the importance of herbs traditionally used for the treatment of infectious diseases, this study is designed to evaluate the antimicrobial activity of *Alpinia galanga* used in the Indian system of medicine for the treatment various diseases. Results showed broad spectrum antimicrobial activity of Methanolic extract of *Alpinia galanga* roots.

Key words: *Alpinia galanga*, Antimicrobial activity, Infectious diseases

Introduction

Natural plant products known as herbal medicines have long been used in control of microorganisms causing plant and human diseases[1]. Medicinal plants are excellent antimicrobial agents because they possess a variety of chemical constituents that are antimicrobial in nature. Recently, much attention has been directed towards extracts and biologically active compounds isolated from popular plant species[2] because of the need for alternative sources of the antibiotics as the pathogenic microbes

are gaining resistance against standard antibiotics[3].

There is thus continuous effort for synthesis of new chemicals having antimicrobial activity. But most of these chemicals are potentially toxic and are not free of side effects on the Host[4].This has urged microbiologist for formulation of new antimicrobial agents and[5-6] evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents[7].

The aim therefore this work to evaluate the antibacterial efficacy of *Alpinia galanga* roots extracts on pathogenic bacterial cultures. *Alpinia galanga* is also known as Greater galangal in English and Kulanjan in Hindi. Most of the South Indian physicians of traditional Ayurveda and Siddha medicine system use *Alpinia galanga* to treat various kinds of disease[8].

Material and Methods

The roots of *Alpinia galanga* was collected from the surroundings of Dehradun city located in Uttarakhand (India). The plant was properly identified and authenticated in the Department of Pharmacognosy Himalaya Wellness Company Dehradun.

Extraction- The roots were collected, washed, air dried in shadow and grinded by mixer grinder. After grinding, 300 gm of plant material was extracted in 1.2 liters of different solvents (methanol, hexane, and water) separately three times at 40°C to 45°C for 6 hours. The organic solvent was filtered by whatman filter paper till clear solution was obtained. Solvent was evaporated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure (vacuum) at 40°C and the semi solid crude extract was placed in a vacuum oven at 40°C for dryness. The crude extra was stored in air tight container at dark place[9].

Screening for Antibacterial and Antifungal Activity-The antibacterial and antifungal (anticandidal) activity was carried out by employing 24h cultures of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Activity of aqueous and methanolic and Hexane extracts of *Alpinia galanga* was tested separately using Agar well diffusion method[10, 11,12,13,14]. The medium was sterilized by autoclaving at 121 ◦C (15 lb/in 2). About 30 ml of the Agar medium with the respective strains of bacteria and fungi was transferred aseptically in to each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 6mm diameter was made using a sterile cork borer. The standard drug and extracts were placed in 6mm diameter well. Antibacterial assay plates were incubated at 37 ± 2˚C for 24h, antifungal(anticandidal) assay plates were incubated at 28 ±2˚C for 48 h. The Ciprofloxacin solution was used as a positive control for antibacterial activity, whereas Clotrimazole was used as positive control for antifungal (anti-candidal) activity, and diameter of the zone of inhibition was measured.

Results and Discussion

Table-1 and 2 showed the antibacterial and antifungal(anticandidal) activity of the crude aqueous, Hexane and methanolic extracts of *Alpinia galanga* on *Staph. aureus, E. coli and Candida*

albicans. The methanolic extract of *Alpinia galanga* showed the highest antibacterial activity with the diameter of zone of inhibition Ranged 15-20mm against *Staph.aureus and E.coli. Hexane and aqueous extract* showed the least range with 10-15 mm as the zone of inhibition while no zone of inhibition observed in aqueous extract against *E.coli*. Against *Candida albicans* methanolic extract showed the highest zone in the range of 15-20 mm followed by aqueous extract in the range of 10-15mm while no activity was detected in Hexane extract as depicted in table-2.

The results obtained in this study revealed antimicrobial efficacy of extracts of *Alpinia galanga* roots. The active components of these plants may be due to their high nonpolar compounds. Methanol extracts were the most potent of all the extracts suggesting that the active component must be a highly nonpolar compound.

The antimicrobial activities of methanolic extracts appeared to be broad spectrum since both the Gram-positive and Gram-negative bacteria were sensitive to their inhibitory effects.

The choice of these microorganisms used in the work was made due to the fact that some of them are causative agents of intestinal, wound and skin infection in human.

Table-1 Antibacterial activity of different extract of *Alpinia galanga*

Test organism	**Diameter of zone of inhibition (mm)**			
	Hexane extract	**Methanol extract**	**Aqueous extract**	**Ciprofloxacin**
E.coli	1+	2+	NAD	3+
Staph. aureus	1+	2+	1+	3+

*1+; 10-15 mm diameter of zone of inhibition 2+; 15-20 mm 3+; 20-25 mm 4+; Above 25 mm

NAD; No Activity Detected

Table-2 Antifungal activity of different extracts of *Alpinia galanga*

Test organism	**Diameter of zone of inhibition(mm)**			
	Hexane extract	**Methanol extract**	**Aqueous extract**	**Clotrimazole**
Candida albicans	ND	2+	1+	3+

*1+;10-15 mm diameter of zone of inhibition 2+;15-20 mm 3+;20-25 mm 4+; Above 25 mm

Conclusion

It was clearly evident from the study that *Alpinia galanga* possess antibacterial/ antifungal properties. When the antibacterial activity of each of the plant extracts were compared for aqueous, Hexane and methanol extracts, significant difference was noticed in their activity. The antibacterial activity of the extracts could be enhanced if the components are purified. These plants

therefore, are potential sources of new drugs for treating infections caused by these clinical pathogens.

Further investigation using bioassay guided fractionation to isolate and characterize the active constituents is under progress.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Pattnaik, M. M.; Kar, M. and Sahu, R. K. Bio-efficacy of some plant extracts on growth parameters and control of diseases in *Lycopersicum esculentum*. *Asian J. of Pant Sci. and Res.*, 2012, 2(2):129-142.
2. Prince, L. and Prabakaram, P. *Asian J. Plant Sci. Res*., 2012, 1:1-84.
3. Tarun, A.; Rachana, S.; Amar, D. S. and Imran, W. *Asian J. Plant Sci. Res*., 2012, 2(1):36 – 40.
4. Geddes, A. M. Prescribes' needs for the developed and third world. In. The Scientific basis of antimicrobial chemotherapy. Greenwood, FO, O' Grady. Editors. Vol. I, *Cambridge University Press.*,1985. P. 265-78.
5. Mamalis P. Some biological properties associated with aminoxy containing compounds. *Xenobiotica.*, 1971,1:569-71.
6. Baregama, L and Talesara, G. L. Synthesis and antimicrobial studies of 3 – alkoxy-59p- substituted ary l0 Biguanidinopentane-2,4 dione and related compounds. *Rese. J. Chem. Enviro*., 2002, 6:59-62.
7. Pandian, M.R.; Banu, G.S. and Kumar, G. A study of the antimicrobial activity of *Alangium salviifolium. Indian J. Pharmacol.*, 2006, 38:203-4.
8. Khare, C. P. A Dictionary of Indian Medicinal Plant. Published by *Springer India Pvt. Ltd.*, 2007, 37.
9. Arya, N.; Chaurasia, S.; Shakya, A.; Bharti, M. and Sahai N. Efficacy of *Ageratum conyzoides* against the control of mosquitoes. *I. J. P. S. R.*, 2011, 2(12):3235-3237.
10. Ruedeekorn, W.; Attawadee, S. Y.; Jiraporn, P.; Chitchamai, O. and Arunporn, I. Development and evaluation of granule and emulsifiable concentrate formulations containing derris elliptica extract for crop pest control. *JAFC.*, 2009, 57:11234-11241.
11. Perez, C.; Paul, M. and Bazerque, P. Antibiotic assay by agar-well diffusion method. *Acta. Biol. Med. Exp.*, 1990, 15:113-115.

12. Murry, P. R.; Baron, E. J.; Pfaller, M. A.; Tenover, F.C. and Yolken, H.R. Manual of clinical Microbiology. 6th edition. ASM Press, *Washington, D. C*., 1995, 15-18.
13. Olurinola, P. F. A Laboratory manual of Pharmaceutical Microbiology. *Idu. Abuja. Nigeria,*1996, 69-105.
14. Srinivasan, D.; Nathan, Sangeetha; Suresh, T. and Lakshmana perumalsamy, P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol.*, 2001, 74:217-220.
15. Greenwood, D.; Slack, R. C.; Peutherer, J. F. Microbiology, 14th edition, Churchill and Living stone, *Spencerswood*, 1992, pp. 1.

About flowers on the cover page

Herbal Non-antibiotics to combat AMR

1. Ocimum sp.-Tulsi

Ocimum sanctum L. (also known as *Ocimum tenuiflorum*, Tulsi) has been used for thousands of years in Ayurveda for its diverse healing properties. Tulsi, the Queen of herbs, the legendary 'Incomparable one' of India, is one of the holiest and most cherished of the many healing and healthy giving herbs of the orient. The sacred basil, Tulsi, is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani system of holistic health and herbal medicine of the East. It is mentioned by Charaka in the Charaka Samhita; an Ayurvedic text. Tulsi is considered to be an adaptogen, balancing different processes in the body, and helpful for adapting to stress. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of 'elixir of life' and believed to promote longevity. Tulsi extracts are used in Ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. Traditionally, *O. sanctum* L. is taken in many forms, as herbal tea, dried power or fresh leaf. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects.

2. Emblica officinalis-Amla / Phyllanthus emblica

Phyllanthus emblica L. (Family: Phyllanthaceae), commonly known as Amla, is arguably one of the most important plants in various traditional and folk systems of medicine in the world. It is used for the treatment of various ailments such as oxidative stress, peptic ulcer, cancer, memory loss, dyspepsia, anemia, heart diseases, and hyperglycemia The fruit is rich in ascorbic acid (vitamin C) and contains several bioactive phytochemicals, of which majority are of polyphenols (ellagic acid, chebulinic acid, gallic acid, chebulagic acid, apeigenin, quercetin, corilaginetc.

3. Alpinia galanga-Kulanjan

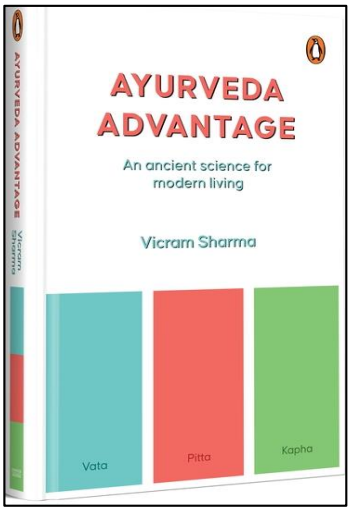
Alpinia galanga (L.) Willd. and *Alpinia officinarum* Hance, commonly known as greater galangal and lesser galangal, respectively, belong to the family of Zingiberaceae (ginger). Due to their spicy flavor and aromatic odors, both of the two rhizomes have long been used as flavoring ingredients and spices in Asia. They also were well-known traditional Chinese medicine and have been widely used as a remedy for gastrointestinal diseases, such as stomachache, dyspepsia, and gastrofrigid vomiting. Previous phytochemical investigations have shown that they have some similar chemical constituents, such as diarylheptanoids, flavonoids, volatile oil, terpenes, phenylpropanoids, and glycosides, but the main chemical components are

different. *A. galanga* is rich in a variety of phenolic compounds and essential oils, whereas *A. officinarum* is rich in flavonoids and diarylheptanoids. Investigations have shown *A. galanga* and *A. officinarum* have many biological activities, including effectiveness as antiinflammatory, antitumor, antiviral, antimicrobial, antioxidant, antiallergic, and gastroprotective agents. This chapter will give an exhaustive review of the botanical properties of these plants. In addition, the phytochemical and pharmacological properties and the adulterant identification of these two plants will also be discussed.

4. Hibiscus rosa-sinensis

Hibiscus rosa-sinensis is one such medicinal plant which has been known to be traditionally used for the management of bronchial asthma. *H. rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5–5 m tall with glossy leaves and red flowers in summer and autumn. Active constituents include β-sitosterol, stigmasterol, taraxeryl acetate, and three cyclopropane compounds and their derivatives, cyanidin glucoside, flavonoids, vitamins, thiamine, riboflavin, niacin, ascorbic acid, quercetin-3-diglucoside, 3,7-diglucoside, cyanidin-3,5-diglucoside, 6-cyanidin-3-sophoroside-5-glucoside, and kaempferol-3-xylosylglucoside. *H. rosa-sinensis* has been shown to have antiinflammatory properties in experimental models of arthritis . Moreover, *H. rosa-sinensis* has traditionally been used for bronchial asthma in most parts of northeastern India). *H. rosa-sinensis* has been shown to contain vital essential nutrients such as calcium, magnesium, zinc, and potassium, which can help in the management of bronchial asthma .

Book review



AYURVEDA ADVANTAGE (An imprint of Penguin Random House)

Vicram Sharma

This book has been authored by Gin Vicram Sharma of the Baidyanath family. It discribes Ayurveda and its advantage in Seventeen Chapters with specific references to Fire of Life, Power of Flavour, Prana and Ojas, Obesity, Herbs, Species, Cleansing within the body, Meditation and Spirituality.

He explains Meditation as a tool to help one empty countless unnecessary thoughts that clutter the mind. According to an article published by National Science Foundation in the US, a person on an average think anywhere between 12000 to 60000 thoughts a day. A study showed that 80 percent of these thoughts are negatively oriented. This means that the mind is feeding the system with a majority of thoughts that are dark, destructive and depressing. Moreover, 75 percent of the thoughts we had the previous day are repeated.

Spirituality is a concept broad and wide enough to hold many different perspectives on the same truth within parameters of a Cosmic ethic. The Spiritual dimension of Ayurveda guides our physiology to connect us with something bigger and beyond the physical.

The author asserts that alignment with Dharma helps one's life flow peacefully and also prays that the holy path of Ayurveda bless our lives with serendipity and transcendence!!!

Dr. I. P. Saxena
Editor

Forthcoming Events

I. **International Conference on Medicinal Plants & Natural Products Research -** Zagazig, Egypt.
July 10, 2024

II. "**10th International Conference on Natural products & Traditional medicine"** - Paris, France.
July 18-19, 2024**.**

III. **34th Annual European Pharma Congress** - Frankfurt, Germany.
July 25-26, 2024.

IV. **8th International Symposium on Phytochemicals in Medicine and Food (8-ISPMF)** - Shenyang, China.
August 02-06, 2024.

V. **4th World Congress on Cardiovascular Medicine Pharmacology** - Paris, France.
September 25-26, 2024

VI. **International Convention The footprint of ethno-pharmacology in drug discovery** - Cape Town, South Africa.
October 23-26, 2024.

VII. **5th European Conference on Natural Products** - Würzburg.
October 22 - 24, 2024

VIII. **37th World Congress on Pharmacology** - Amsterdam, Netherlands.
October 31-01, 2024

IX. **10th International Conference on Antimicrobials & Antibiotic Resistance-** Vancouver, Canada. November 25-26, 2024

X. **7th International Pharmacy and Pharmaceutical Conference** - Rome, Italy.
November 28-29, 2024

XI. **17th World Drug Delivery Summit** - Dubai, Uae.
December 05-06, 2024.

XII. **International Conference on Natural Products (ICNP) -** Tokyo, Japan
December 02, 2024

XIII. **International Conference on Natural Products (ICNP)** - Barcelona, Spain
December 23, 2024

XIV. **International Conference on Molecular Biology, Biochemistry and Biotechnology (ICMBBB)** - Paris, France
November 18, 2024

XV. **International Conference on Molecular Biology, Biochemistry and Biotechnology (ICMBBB)**- Bangkok, Thailand
November 25, 2024

Instructions to Contributor

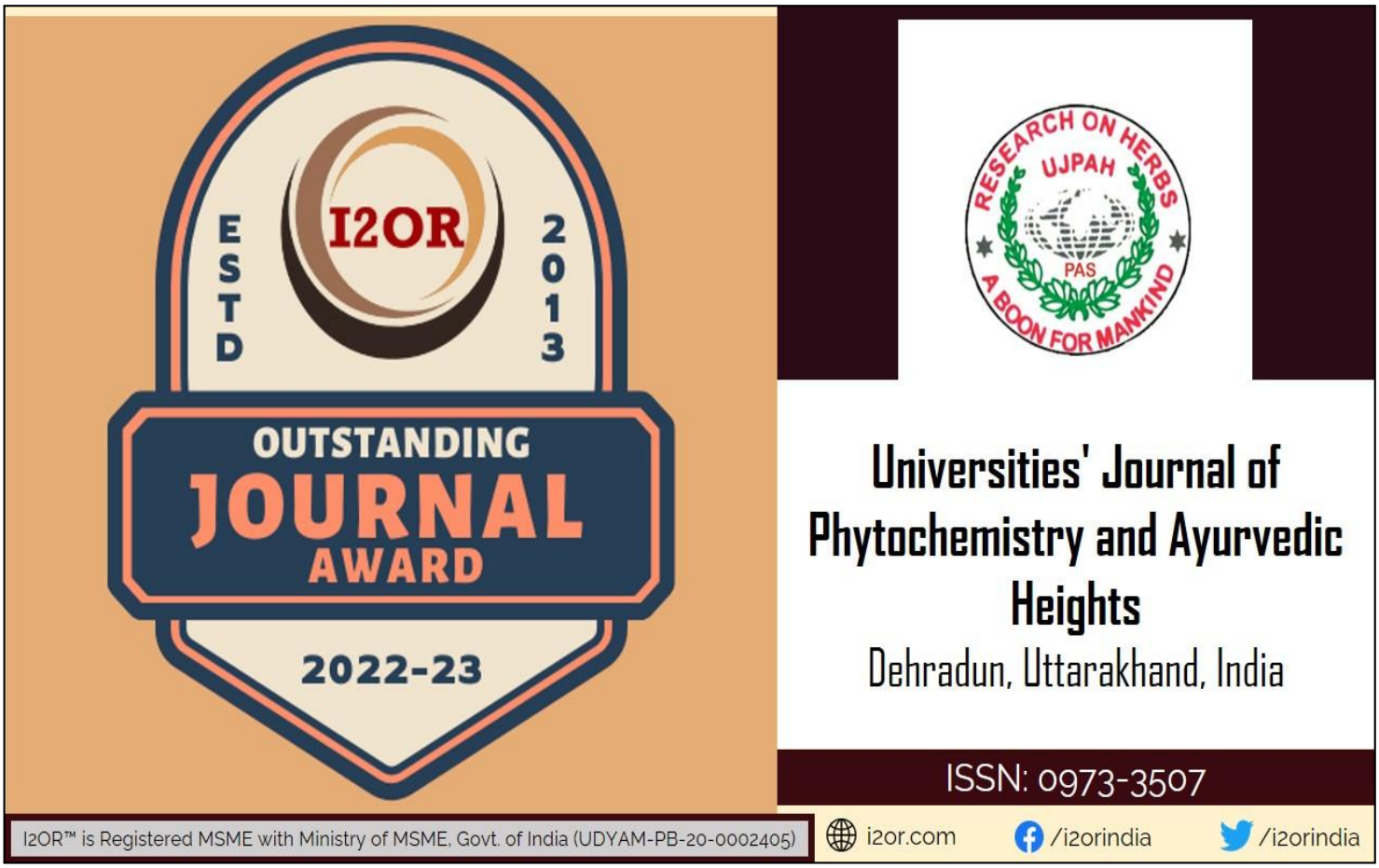
The Universities' Journal of Phytochemistry and Ayurvedic Heights is a bi-annual Journal publishing free of charge the research work on herbs, natural products, phytochemicals and indigenous traditional system on Indian medicines for Human Health Protection, Nutrition and Plant Defence. It is open to research workers-India and abroad. Original articles, Research articles, short communications and Letters to the Editor on relevant topics are published-English. The copyright lies with the publisher. Publication of paper will be-clear understanding that they have not been submitted for publication-any other journal.

The Journal has a Helping Board which assists young researchers free of charge to write article-the prescribed format, modify and correct it.-such case such paper/s may be addressed to 'The Director Helping Board', Universities' Journal of Phytochemistry and Ayurvedic Heights, 1-Inder Road, Dehradun, UK. The papers are the only accepted for publication on the advice of the referees.

1. The typed manuscript should not exceed 10 pages and tables and graphs should not exceed 10% of the typed manuscript.
2. Manuscript should be typed on one side of the paper with double spaces. The usual format of a manuscript comprises of Abstract (not exceeding 150 words), Introduction, Keywords, Material and Methods, Observations, Results, Discussion, Acknow-ledgements, Disclaimer, References and Tables & Figures.
3. Tables & Figure should be double spaced on separate pages, numbers consecutively-Roman numerals and placed at the end of manuscript.
4. Those students who want their papers to be evaluated, corrected and formatted may address to the Director Helping Board.
5. The reprint of their articles will be supplied to the authors (First author) free of charge. Orders for additional reprints should be made along with the manuscript. They will be charged for postage and packing.
6. It is emphasized that the manuscripts should be neatly typed, double spaced throughout including tables on pages of uniform size with at least 2.5 cms margin on all sides. Author(s) should not break or hyphenate the words. Authors are required to include:
   I. The chemical structure, formula and proprietary name of novel or ill defined compounds.
   II. The w/w yield of prepared extracts-terms of starting crude material.
   III. Complete formulation details of all crude drug mixtures.
   IV. The herbarium specimen number of plant(s) studied-case of less well known plants be cited using the collector and collection number (eg. Doe 123) as well as indicating the names of the herbarium institution where it has been deposited.
7. **Keywords:** Authors are requested to assign 3-6 keywords to the manuscript preferably taken from index Medicus of ExcerptMedica Index for abstracting and indexing purposes. They should be mentioned after abstract and before Introduction of the Manuscript.
8. References to literature are to be arranged alphabetically or with superscript number as given-the manuscript wise and to be placed at the end of the article and referred to by name and year chronologically. Standard abbreviations listed-the World list of Science Periodicals should be used. A sample citation is given here:

Singh,G.; Dhar, U. and Singhal, A.K. Origin of Kashmir Saffron – a possible clue from weeds. *Sci.Cult*. 2005, 43(1): 97-102.

9. Contributors are requested to send their manuscripts via email. The software for text should preferably be MS Word for Windows version 6.0 onwards. Corel Draw or any other compatible software or as picture-MS Word may be used for giving illustrations. Images and PDF files are not acceptable.
10. Authors should fill-the copy right form and give undertaking that the research article/s they are sending has not been published elsewhere and that no unethical practice has been adopted.
11. Research papers and correspondence pertaining to Journal be sent on the following addressdr.sfarooq.him@gmail.com/ editor@ujpah.in


ESTD 2013
I2OR
OUTSTANDING
JOURNAL
AWARD
2022-23
RESEARCH ON HERBS
UJPAH
PAS
A BOON FOR MANKIND
Universities' Journal of Phytochemistry and Ayurvedic Heights
Dehradun, Uttarakhand, India
ISSN: 0973-3507
I2OR™ is Registered MSME with Ministry of MSME, Govt. of India (UDYAM-PB-20-0002405)
i2or.com
/i2orindia
/i2orindia


ESTD 2013
I2OR
OUTSTANDING
JOURNAL
AWARD
2022-23
AWARD
CERTIFICATE
presented to
Universities' Journal of Phytochemistry and Ayurvedic Heights
ISSN: 0973-3507
I2OR™ is Registered MSME with Ministry of MSME, Govt. of India (UDYAM-PB-20-0002405)
Dr. S.N. Mehta
Director
I2OR India
I2OR
Er. T. Singh
Head (R&D)
I2OR India
I2OR/2022-23/PEA-004

Notes

WITH BEST COMPLIMENTS

from

Tasmia All India Educational & Social Welfare Society

1-Inder Road, Dehradun-248001 (UK.)

Our Mission: Education for All.
upliftment of weaker section and
to save environment.

LET US MAKE OUR CONTRIBUTION TO SAVE OUR PLANET

Educate and motivate each other to care for the environment.

Protect trees – Use Minor Forest Produce only.

Plant trees – one grown tree can clean out air pollution generated by one heavy Transport vehicle. Medical tree plantation will care for Health and will enrich environment.

Contact for sapling at email:dr.sfarooq.him@gmail.com

Website: www.tasmia.in

DR. S. FAROOQ
President

Uttarakhand State Council for Science & Technology

Department of IT, Suraj and Science Technology

(Govt. of Uttarakhand)
Vigyan Dham, Jhajra, Dehradun- 248015
and- PH. +91-135-2976266
Email: ucost@ucost.in

Being a hilly state with limited technological interventions in difficult terrains, the Science and Technological advancement have not received much attention so far. Therefore, Uttarakhand State Council for Science and Technology (UCOST) is playing a crucial role in developing S&T driven ecosystem for the state. The council has initiated the establishment of a Science City which will be fifth in the nation, Regional Science and Innovation Centers in all 13 districts of the state. For the promotion of Science, Engineering, and Mathematics (STEM) labs will be established at block level and the council has already initiated Labs on Wheels to bring S&T to school-level. The Children Science Congress and Seemant Bal Vigyan Mahotsava, along with the Gyanotkarsh program also newly initiated by the council to develop S&T ecosystem at the school and higher education level. The Council aims to forge conducive, result-oriented partnerships between central and state government, civil society organizations, R&D Institutions, Academia and Industry. The Regional Science Centre within the UCOST campus having science exhibits along with innovation lab sponsored by the National Innovation Council drawing large crowds from schools and colleges.

Phytochemistry provides a crucial lens through which we can decipher the therapeutic potential of Ayurvedic herbs. These natural compounds, meticulously identified and studied, unveil the intricate mechanisms behind traditional remedies that have nurtured health and well-being for millennia. In our exploration, we celebrate not only the potency of phytochemicals but also the holistic principles of Ayurveda. This ancient system, rooted in harmony and balance, offers profound insights into personalized medicine and preventive care- an approach increasingly relevant in our dynamic world.

I extend my best wishes to the Universities' Journal of Phytochemistry and Ayurvedic Height for their endeavor in herbal research.

Prof. Durgesh Pant
Director General