ORIGINAL ARTICLE

Study for the preparation of medicinal herbal extracts from *Thymus vulgaris*, *Rosmarinus officinalis* L., and *Origanum vulgare* L. for its health-care applications

N. Budhiraja[1], N. Singh[1], S. Chaubey[1], S. Ojha[1], R. Kumar[2], G. Kaur[3], S. Peta[4], S. Vats[1]*

[1]Faculty of Biotechnology, Institute of Bio-Sciences and Technology, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India, [2]University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra, India, [3]Faculty of Biosciences, Institute of Bio-Sciences and Technology, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India, [4]Faculty of Chemical Sciences, Institute of Natural Sciences, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India

ABSTRACT

Aim: This study aims to extract the phytochemicals from thyme, rosemary, and oregano and evaluate their shelf life and antimicrobial properties. **Introduction:** The world has witnessed unprecedented time with another virus-based pandemic, severe acute respiratory syndrome coronavirus 2 or novel coronavirus disease 2019. A good immune system is the ultimate shield toward pathogenic diseases. Use of natural and herbal products and extracts to boost immune system has gained momentum. Phytochemicals present in the herbs, spices, and plants have positive impact on fight against pathogens. **Materials and Methods:** This study has taken singular and synergistic effect of the *Thymus vulgaris*, *Rosmarinus officinalis* L., and *Origanum vulgare* for health-care application. Extraction from the herbs was done with the two methods low temperature for long time and high temperature for short time. **Results and Discussion:** In the present study, qualitative study of the phytochemicals present in the each sample was determined, antimicrobial activities and shelf life study were carried out. **Conclusion:** The present study open door for the application of phytochemical extracts against new emerging pandemics related to microbes present in air, water and soil. All the three herbs have been traditionally used in the medicinal systems. A detailed study can reveal the individual and synergistic applications of phytochemicals.

Key words: Antimicrobial, Phytochemicals, *Rosmarinus officinalis* L. and *Origanum vulgare*, *Thymus vulgaris*

INTRODUCTION

Spices have long been used for flavoring, coloring, and preserving food, as well as for medicinal purposes, and serve as an integral part of culinary culture around the world. In addition, many of the spices are proven to protect against the development of acute and chronic, non-communicable diseases, and help people maintain health.[1-3] There is now sufficient evidence that spices and herbs possess antioxidant, anti-inflammatory, anti-tumorigenic, anticarcinogenic, antimalarial, antifungal, and glucose- and cholesterol-lowering activities. Herbs such as rosemary are excellent sources of antioxidants with their high content of phenolic compounds.[3,4-7] Most of the medicinal plants are an upscale source of novel drugs that form the ingredients in traditional medicinal systems, modern medicinal products, nutraceuticals, food supplements, folk medicines, medicines intermediate bioactive principles, and lead compounds in synthetic drugs. Humankind has been using plant medicines since ages for the diagnosis and cure of various diseases. They are the best possible tools to tackle

Address for correspondence:
Dr. Siddharth Vats, Faculty of Biotechnology, Institute of Bio-Sciences and Technology, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India. Phone: +91-9140951132. E-mail: vatssidd@gmail.com

Received: 19-04-2021
Revised: 26-08-2021
Accepted: 06-09-2021

the disease, as they have the lowest possible side effects compared to other available forms of drugs and are used to treat the diseases.[8,9] Naturally occurring compounds have a range of chemical diversity including antiviral activity and can, therefore, be useful as therapeutic agents against viral infections. Antiviral drugs represent the first line of defense to a novel strain of pandemic infection. As estimated by a World Health Organization, more than 80% of the world's population depends on conventional plants to meet their health needs.[10-13] It is well known that the natural activity of plants is due to the presence of secondary metabolites which are produced from the plant cell in small amount, in specific parts of plant, and in the specific period of plant growth. Secondary metabolite production *in vitro* is possible through cultivation of plant tissue. Plants derived from tissue production can be a source of valuable natural products.[14].The aggregation of secondary products in crops of plant cells depends on several factors including the composition of the medium of culture and environmental conditions.[15,16] *Thymus vulgaris, Rosmarinus officinalis* L.*,* and *Origanum vulgare* are therapeutically important medicinal plants, among herbs and spices. *R. officinalis* L. is a common domestic plant cultivated in many parts of the world. It is used in traditional medicines for its choleretic, hepatoprotective, and anti-tumorigenic activities, and is also used to flavor food, cosmetics, and herbal medicine.[17,18] The majority of rosemary extracts are related to their content of mainly diterpenes (e.g. carnosic acid) active constituents; phenolic acids (e.g. rosmarinic acid); and flavonoids were derived from two common flavones: Apigenin and luteolin. Rosemary is highly esteemed for its herbal, antioxidant, antimicrobial, or antitumoral properties. Rosemary extracts exhibit a strong antioxidant activity associated with the presence of substances derived from secondary metabolism, mainly phenolic compounds.[19-23] Rosemary extracts possess marked antibacterial, antifungal, and antiviral properties and activity against certain bacteria including *Staphylococcus aureus*, *Staphylococcus albus*, *Vibrio cholerae*, *Escherichia coli*, and *Corynebacteria* spp. and yeast including *Candida albicans.*[24,25] On the other hand, oregano (*O. vulgare* L.*)* is a therapeutically important medicinal plant, among herbs and spices. Oregano is a common domestic plant cultivated in many parts of the world. It is used in traditional medicines for its choleretic, hepatoprotective, and anti-tumorigenic activities, and is also used to flavor food, cosmetics, and herbal medicine. *O. vulgare* L. have high antioxidant activity. Thymol and carvacrol present in oregano plant have antioxidant activity higher than many known antioxidants.[26,27] Phytochemicals in *O. vulgare* L. show antibacterial properties, these include carvacrol, β-fenchyl alcohol, thymol, and γ-terpinene.[28,29] Similarly, thyme belongs to the family Lamiaceae and, till now, 928 species of the genus. Thymus has been identified in Europe, North Africa, Asia, South America, and Australia. Among these species, *T. vulgaris* (common thyme, German thyme, thyme) is commonly used as a culinary herb and it also has a long history of use for different food and medicinal purposes.[26] On the basis of GC and GC–MS analysis of thymes, essential oils researchers have identified 41 components that account 97.85% of the total known constituents. The major constituents of thymes essential oil are camphor (39.39%), α-pinene (9.55%), camphene (17.57%), 1,8-cineole (5.57), β-pinene (4.32%), and borneol (5.03%). The work here proposes that the synergistic activity of *T. vulgaris*, *R. officinalis* L.*,* and *O. vulgare* should be studied as a potential agent against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and related virus and that it can be a potential resource for novel COVID-19. Since December 2019, the novel coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2, has been in the midst of global hysteria and health concern. The World Health Organization (WHO) reported on March 26, 2020, that 416,686 and 18,589 death cases were confirmed worldwide and it has spread to 197 countries.[30] At this point, understanding the virus basic mechanism for developing specific drugs is vital. SARS-CoV-2 currently shares sequence homology with SARS-CoV and a bat coronavirus.[31] Despite its similarity to SARS-CoV, its transmission efficiency and diagnostic methods are rather different. The distinguishing factor is probably the nucleotide changes in the spike (S) protein and its receptor-binding domain (RBD).[32-34] The treatments currently include lopinavir/ritonavir and supportive care; as this depends primarily on the severity of the disease. Different drugs are being produced at an extremely rapid and new targets are identified regularly, and several drugs are now being evaluated in clinical trials. Researchers are very curious about how to give the best public safety before a vaccine can be made available. Medicinal herbs are a promising field for the treatment of various illnesses.[30] By identifying certain phytocompounds, it is possible to effectively characterize medicinal herbs that could help to alleviate the infection. These spices have long been used for flavoring, coloring, and preserving food, as well as for medicinal purposes, and serve as an integral part of culinary culture around the world. In addition, many of the spices are proven to protect against the development of acute and chronic, non-communicable diseases and help people maintain health. There is now sufficient evidence that spices and herbs possess antioxidant, anti-inflammatory, anti-tumorigenic, anticarcinogenic, and glucose- and cholesterol-lowering activities. Herbs such as rosemary are excellent sources of antioxidants with their high content of phenolic compounds.[35]

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and solvents used were obtained from Titan Biotech Limited from local distributor. Acetone, ethanol, nutrient media, Mueller-Hinton agar (MHA), etc., were of quality standard. Glassware was also procured from Titan Biotech.

Collection and Plant Material Identification

Dried whole leaves of *T. vulgaris*, *R. officinalis* L., and *O. vulgare* plants were used for this experiment and were bought from M.M. Enterprise, and they are known to provide finest quality products and high standard services to the buyers. This product was delivered by Amazon agent to our doorsteps on January 30, 2020.

Pre-treatment of Plant Material

The collected plant materials were dried in oven at 60^0C for 30 minutes to remove the moisture content present in them. After drying in oven and removing all the moisture content as per the NREL Protocol, the leaves was grinded using a mechanical grinder to form fine powder material of the dried leaves. All the three plants were grinded separately. After grinding, the powder formed was sieved through sieve tube so that any bigger particle is not present in the powder and the powder has fine texture. After sieving, the obtained powder was then kept in an airtight container to avoid any moisture contact with the leaf powder as per the protocols provided by Vats *et al*. (2017).[4]

Preparation of Phytochemical Extract of Plant Material

For the extraction of phytochemicals from powder of *T. vulgaris*, *R. officinalis* L., and *O. vulgare,* two criteria that are high temperature short time (HTST) and low temperature and high time (LTHT) based on temperature and time were used. The prepared leaf powder was weighed and mixed with solvents. The solvents were chosen on the basis of literature reviewed. The literature helped us to focus on the phytochemicals and the solvent in which they are extractable was focused on.

Extract Preparation at Low Temperature for Long Time (LTLT)

Cold extract of the sample at LTLT was prepared by keeping 5 g of *T. vulgaris, R. officinalis* L., and *O. vulgare* leaf powder in 30 mL of each solvent separately. This mixture was kept sealed in a conical flask for some days at room temperature. Then, the sample as filtered and stored at 4°C for further use and the filtrate was dried and its dry eight as measured.

Extract Preparation at High Temperature for Short Time (HTST)

The Soxhlet extract of the sample at high temperature of short time was prepared by taking 5 g of powdered sample of each *T. vulgaris*, *R. officinalis* L., and *O. vulgare* in the thimble. After that, the thimble was placed in Soxhlet apparatus and the apparatus was run with each sample at a temperature slightly lower than their boiling temperature until the solvent takes all the phytochemicals present inside the sample. Five cycles were run for each sample of each plant. Then, the accumulated solution in round bottom flask was left to cool after cooling was stored at 4^0 C.

Preparation of Microbial Culture and Plates for Test

Microbes taken in this study are from air, water, and soil. A stock solution was prepared from the microbes that were able to grow on the nutrient agar plates on to which they were collected. These microbes were then used for the further studies. Microbes from air, water, and soil were stocked separately in different plates. Microbes from stock culture were inoculated in sterilized and autoclaved Mueller-Hinton broth (MHB) to the conditions mentioned on the box. Fifteen grams of MHB were dissolved in 1000 ml flask containing 500 ml of distilled water, pH 7.0 and incubated for 24 hours at 28 ± 2^0C in an incubator shaker at 120 rpm. Plates were prepared of Mueller-Hinton agar (MHA) to check the antimicrobial activity of filter paper disc coated with phytochemicals extracts of *T. vulgaris, R. officinalis* L., and *O. vulgare*. Discs were 8 mm in size and prepared from sterilized filter paper.

Assay for Antimicrobial Activity

Modified Bauer–Kirby disc diffusion method was followed to study antimicrobial activity of silver nanorods particle.[5,6,36,37] Discs used were made up of sterilized filter paper and had a diameter of 8 mm. These discs were then impregnated with 0, 20, 30, and 50 µl of phytochemical extract and were placed onto Mueller-Hinton agar (MHA) plates made up of autoclaved MHA media and had bacteria swabbed (100 µl). These plates were then incubated overnight at 28 ± 2°C and the zone of inhibition around the discs was measured. Large zones of inhibition around the disc indicated susceptibility of microbe toward that phytochemical extracts, while small zones or no zones of inhibition indicated resistive microbes.

Estimation of Shelf Life of Phytochemicals Extracts of *T. vulgaris*, *R. officinalis* L., and *O. vulgare*

The shelf life of extracts obtained as estimated based on their antimicrobial activity as per time period. The phytochemical rich extract is considered to be stable and of high shelf life if it has antimicrobial activity maintained. A shelf life is the time period taken by a product to decay 90% of its original activity.[38] Antimicrobial activities of the extracts against microbe present in air, water, and soil. This was done by preparing Mueller-Hinton agar media and then its inoculation with general microflora present in air, water, and soil. After preparing media, plates were divided into four parts and marked one as control and rest three as different phytochemical extracts for *T. vulgaris, R. officinalis* L., and *O. vulgare,* respectively. Then, discs of phytochemical extracts were prepared and inserted on media according to their markings. All prepared plates were sealed up and kept in BOD incubator for 24 h to see the results. The results were analyzed as per Ojha *et al*., 2013.[5,6]

Qualitative Estimation of the Various Extracts Prepared

The extracts prepared were then sent to SAIF/CIL Panjab University, Chandigarh, for qualitative estimation from GC–MS.

RESULTS AND DISCUSSION

Extract preparation at LTLT and HTST was subjected to column chromatography as extract produced contain different group of phytochemicals, purified, and separated into polar and non-polar components using silica gel chromatography. Silica gel retains polar phytochemicals because of silanol groups. During separation of phytochemicals, column elution was performed with solvents with increasing polarities. The samples obtained were sent to SAIF/CIL, Chandigarh, for qualitative estimation. Cold extract of the sample at LTLT was prepared and results are shown in Tables 1-3, where the individual results for both powdered and non-powdered sample's weight loss, before solvent extraction, and post-solvent extraction are shown. Similarly, results for extract preparation at HTST are shown in Tables 4-6.

The solvents ethanol, methanol, and water used in our study have already been reported to be used for the extraction of phytochemicals from plants which have antimicrobial properties by many of the researchers. The Soxhlet extract of the sample at high temperature of short time was prepared by taking 5 g of *T. vulgaris, R. officinalis* L., and *O. vulgare* sample, respectively, and separately in the thimble. For each sample, thimble filled with the leaves and powder, was placed in Soxhlet apparatus and the apparatus was run with each sample at a temperature slightly lower than their boiling temperature until the liquid present inside it becomes colorless to colored. Then, the accumulated solution in round bottom flask was left to cool after cooling was stored at 4°C. Tables 4–6 are showing the preparation of HTST extracts of *T. vulgaris, R. officinalis* L., and *O. vulgare,* respectively. Different extracts were prepared for each plant of *T. vulgaris*, *R. officinalis* L., and *O. vulgare,* respectively. Extracts were prepared based on time period, temperature, and variability of solvents (acetone, ethanol, water, and methanol). Tables 1-3 show the weight difference in the sample pre- and post-extraction in LTLT. It is an indication of release of phytochemicals present in the samples. For *R. officinalis* L., acetone is the best solvent for the release of phytochemicals based on weight loss. Similarly for *T. vulgaris* and *O. vulgare*, water and acetone are the suitable solvents based on weight loss. For HTST**,** weight loss post-extraction for *T. vulgaris*, *R. officinalis* L.*,* and *O. vulgare* was in ethanol solvent system for all.

Estimation of Shelf Life of Phytochemicals Extracts of *T. vulgaris, R. officinalis* L., and *O. vulgare*

The shelf life of extracts obtained as estimated by checking their antimicrobial activity against microbes of air, water, and soil. This was done by preparing Mueller-Hinton agar media with air, water, and soil pathogens or microbes separately. After preparing media, plates were divided into four parts and marked one as control and rest three as different phytochemical extracts. Then, discs of phytochemical extracts were prepared

Table 1: Extract preparation at LTLT for *R. officinalis L.*

Type of extract	Type of solvent		No. of days it was kept dissolved (Days)	Temp. At which it was kept (room temp. °C)	*R. officinalis L.* leaves weight (g)		*R. officinalis L.* leaf powder weight (g)	
	Polar	Non-polar			Before	After	Before	After
Acetone	Yes	Yes	06	22–27	-	-	5.001	3.717
Ethanol	Yes	No	13	22–27	5.090	3.850	5.016	3.940
Water	Yes	No	12	22–27	5.035	3.673	5.032	3.851

R. officinalis: *Rosmarinus officinalis*, LTLT: Low temperature for long time

Table 2: Extract preparation at LTLT for *T. vulgaris*

Type of extract	Type of solvent		No. of days it was kept dissolved (Days)	Temp. at which it was kept (room temp. °C)	*T. vulgaris* leaves weight (g)		*T. vulgaris* leaf powder weight (g)	
	Polar	Non-polar			Before	After	Before	After
Acetone	Yes	Yes	06	22–27	-	-	5.042	3.827
Ethanol	Yes	No	13	22–27	5.080	3.940	5.028	3.961
Water	Yes	No	12	22–27	5.005	3.643	5.078	3.811

T. vulgaris: *Thymus vulgaris*, LTLT: Low temperature for long time and inserted on media according to their markings. All prepared plates were sealed up and kept in BOD incubator for 24 h to see the results. Tables 7–9 show the antimicrobial activity based shelf life study of extracts of *T. vulgaris*, *R. officinalis* L., and *O. vulgare,* respectively. From the study, it can be observed that phytochemical extracts have antimicrobial activity against general microflora present in air, water, and soil.

Qualitative Estimation of the Various Extracts Prepared

The samples sent to SAIF/CIL Panjab University, Chandigarh, were estimated and Table 10 shows the phytochemicals present in the samples of *T. vulgaris, R. officinalis* L., and *R. officinalis* L. sample contained caffeic acid, medioresinol, p-coumaric acid, luteolin-rutinoside, luteolin-hexoside, isorhamnetin-3-o-hexoside, 4-hydroxybenzoic acid, apigenin-7-O-glucoside, hesperidin, homoplantaginin, rosmarinic acid, rosmanol methyl ether, rosmadial or rosmanolquinone, rosmanol methyl ether isomer, rosmadial, and rosmaridiphenol. *O. vulgare* found to contain 7-methyl-epirosmanol, carnosol, rosmanol, carnosic acid, epirosmanol, isorosmanol, etc. Similarly, *T. vulgaris* found to contain alpha pinene; alpha terpineol; caffeic acid; camphor; carvacrol; carvacrol methyl ether; caryophyllence; caryophyllence oxide; cineole (slightly); endo-borneol; geranyl acetate; gerniol; limonene; linalool; myrcen; oleanic acid; p-cymene;

Table 3: Extract preparation at LTLT for *O. vulgare*

Type of extract	Type of solvent		No. of days it was kept dissolved (Days)	Temp. at which it was kept (room temp. °C)	***Origanum vulgare* leaves weight (g)**		***Origanum vulgare* leaf powder weight (g)**	
	Polar	Non-polar			Before	After	Before	After
Acetone	Yes	Yes	06	22–27	-	-	5.064	3.312
Ethanol	Yes	No	13	22–27	5.040	3.939	5.027	3.962
Water	Yes	No	12	22–27	5.003	3.642	5.079	3.852

*O. vulgare: Origanum vulgar*e, LTLT: Low temperature for long time

Table 4: Extract Preparation at HTST for *R. officinalis L.*

Type of extract	Type of solvent		Temperature at which Soxhlet apparatus was run (°C)	Conc. of solvent used (%)	***R. officinalis* L. leaf powder weight (g)**	
	Polar	Non-polar			Before	After
Ethanol	Yes	Yes	60	70	5.047	3.495
Methanol	Yes	No	55	40	5.076	3.725
Water	Yes	No	90	100	5.028	3.872

R. officinalis: Rosmarinus officinalis, HTST: High temperature for short time

Table 5: Extract preparation at HTST for *T. vulgaris*

Type of extract	Type of solvent		Temperature at which Soxhlet apparatus was run (°C)	Conc. of solvent used (%)	***T. vulgaris* leaf powder weight (g)**	
	Polar	Non-polar			Before	After
Ethanol	Yes	Yes	60	70	5.039	3.593
Methanol	Yes	No	55	40	5.066	3.826
Water	Yes	No	90	100	5.073	3.757

T. vulgaris: Thymus vulgaris, HTST: High temperature for short time

Table 6: Extract preparation at HTST for *O. vulgare*

Type of extract	Type of solvent		Temperature at which Soxhlet apparatus was run (°C)	Conc. of solvent used (%)	***O. vulgare* leaf powder weight (g)**	
	Polar	Non-polar			Before	After
Ethanol	Yes	Yes	60	70	5.040	3.490
Methanol	Yes	No	55	40	5.000	3.645
Water	Yes	No	90	100	5.013	3.678

O. vulgare: Origanum vulgare, HTST: High temperature for short time

Table 7: Antimicrobial activities of phytochemicals present in the extract of *R. officinalis* L.

Type of extract	Extract of solvent	Antimicrobial activity in freshly prepared extracts			Antimicrobial activity in extracts kept at room temperature for 30 days		
		Air	Water	Soil	Air	Water	soil
Cold extract	Ethanol extract (*R. officinalis* L. leaves)	6 mm	6.5 mm	7.5 mm	5 mm	5.5 mm	6 mm
	Ethanol extract (*R. officinalis* L. leaf powder)	5 mm	2 mm	3 mm	4 mm	1.5 mm	2 mm
	Acetone extract (*R. officinalis* L. leaf powder)	3 mm	6 mm	5 mm	2.5 mm	5 mm	4.5 mm
	Water extract (*R. officinalis* L. leaves)	11 mm	4 mm	5 mm	9.5 mm	3 mm	4 mm
	Water (*R. officinalis* L. powder extract leaf	7.5 mm	2 mm	5 mm	7 mm	1 mm	4.5 mm
Soxhlet extract	Ethanol (*R. officinalis* L. powder) extract leaf	4 mm	2 mm	1 mm	3.5 mm	1 mm	1 mm
	Methanol (*R. officinalis* L. powder) extract leaf	2 mm	2 mm	2 mm	1 mm	1 mm	1.5 mm
	Water (*R. officinalis* L. powder) extract leaf	2 mm	8 mm	2 mm	2 mm	7 mm	1 mm

R. officinalis: *Rosmarinus officinalis*

Table 8: Antimicrobial activities of phytochemicals present in the extract of *T. vulgaris*

Type of extract	Extract of solvent	Antimicrobial activity in freshly prepared extracts			Antimicrobial activity in extracts kept at room temperature for 30 days		
		Air	Water	Soil	Air	Water	Soil
Cold extract	Ethanol extract (*T. vulgaris* leaves)	6 mm	6 mm	8 mm	5 mm	5 mm	6.5 mm
	Ethanol extract (*T. vulgaris* leaf powder)	4 mm	2 mm	3 mm	3 mm	1.5 mm	2 mm
	Acetone extract (*T. vulgaris* leaf powder)	3 mm	7 mm	5 mm	2.5 mm	6 mm	4 mm
	Water extract (*T. vulgaris* leaves)	12 mm	2 mm	5 mm	10.5 mm	1 mm	4 mm
	Water extract (*T. vulgaris* leaf powder)	7 mm	4 mm	5 mm	6.5 mm	3 mm	4.5 mm
Soxhlet extract	Ethanol extract (*T. vulgaris* leaf powder)	4 mm	2 mm	1 mm	3.5 mm	1 mm	1 mm
	Methanol extract (*T. vulgaris* leaf powder)	2 mm	2 mm	2 mm	1 mm	1 mm	1.5 mm
	Water extract (*T. vulgaris* leaf powder)	2 mm	8 mm	2 mm	2 mm	7 mm	1 mm

T. vulgaris: *Thymus vulgaris*

rosemeric acid; sabinene; thymol; triterpene; y-terpinene; 4-terpineol, etc. These phytochemicals are responsible for their various health-care applications. Rios and Reco, 2005**,** observed that the interests of scientific community on health-care applications of phytochemicals have been continuously increasing.[39,40] There is wide range of criteria that have been studied by the researchers. Many of them have focused on the antimicrobial properties of various extracts obtained from various parts of the plants based on traditional knowledge, folk medicines, essential oils, compounds isolated from plants, and various groups of phytochemicals such as flavonoids, terpenes, alkaloids, proteins, essential oils, and flavones. Vats and Miglani, (2011), studied the synergistic antimicrobial effects of cow urine and *Azadirachta indica* on Gram-positive and Gram-negative microbes.[2] The result obtained by their study shows that *Azadirachta indica* has phytochemicals which can inhibit the growth of the microbes. Moreover, in the presence of photoactivated cow urine, their antimicrobial activity got increased. Ojha *et al*., (2013), studied the antimicrobial activity of the nanoparticles, based on the aspect ratio.[5,6] However, there is always some toxicity associated with the nanoparticles which can be overcome by the application of phytochemicals.[37,38,41,42] Vats (2017) has explained the various methods and strategies where phytochemicals can be extracted from forest biowastes.[4] In this study, the author has also mentioned the

Table 9: Antimicrobial activities of phytochemicals present in the extract of *O. vulgare*

Type of extract	Extract of solvent	Antimicrobial activity in freshly prepared extracts			Antimicrobial activity in extracts kept at room temperature for 30 days		
		Air	Water	Soil	Air	Water	Soil
Cold extract	Ethanol extract (*O. vulgare* leaves)	6 mm	6 mm	8 mm	5 mm	5 mm	7 mm
	Ethanol (*O. vulgare* powder) leaf extract	3 mm	2 mm	3 mm	3 mm	1.5 mm	2 mm
	Acetone (*O. vulgare* powder) leaf extract	3 mm	7 mm	4 mm	2.5 mm	5 mm	3 mm
	Water extract (*O. vulgare* leaves)	9 mm	4 mm	3 mm	10.5 mm	1 mm	4 mm
	Water (*O. vulgare* powder) leaf extract	5 mm	4 mm	5 mm	6.5 mm	3 mm	4.5 mm
Soxhlet extract	Ethanol (*O. vulgare* powder) leaf extract	3 mm	2 mm	2 mm	3.5 mm	2 mm	1 mm
	Methanol (*O. vulgare* powder) leaf extract	2 mm	4 mm	2 mm	1 mm	1 mm	1.5 mm
	Water (*O. vulgare* powder) leaf extract	2 mm	8 mm	4 mm	2 mm	5 mm	2 mm

O. vulgare: Origanum vulgare

various techniques and methods associated with optimum extraction of phytochemicals for health-care applications. *O. vulgare* and *T. vulgaris* oils demonstrated bacteriostatic effect against five Gram-positive and eight Gram-negative bacterial strains due to the antibacterial properties of thymol and carvacrol contained in the oils. The essential oil of *O. vulgare* had substantial antimicrobial activity against 10 bacteria, 15 fungi, and a yeast species. The antibacterial activity of rosemary has been previously exhibited in various assay types based on either MIC or MBC. In this regard, Sienkiewicz *et al.* demonstrated the antibacterial activities of rosemary (*R. officinalis* L.) against *E. coli*. Other studies have also shown the antibacterial activity of rosemary oil against *E. coli*, *Bacillus cereus*, *S. aureus*, *S. aureus*, *Clostridium perfringens*, *Aeromonas hydrophila*, *B. cereus,* and *Salmonella choleraesuis*. This essential oil was incorporated into meat reporting antibacterial activity against *Brochothrix thermosphacta* and Enterobacteriaceae. Studies done by Sales *et al*. had confirmed parallel results.[43-46]

This study is also the continuation of the same.[16] Vats *et al*., (2012), also studied the minimum inhibitory concentration of photoactivated cow urine, *Azadirachta indica*, *Terminalia chebula,* and *Piper nigrum* against *Candida glabrata* (MTCC 3019), *Streptococcus mutans* (MTCC 497), *Streptomyces aureofaciens* (MTCC 325), *Pseudomonas aeruginosa* (MTCC 7093), *Candida parapsilosis* (MTCC 1965), *C. albicans* (MTCC183), *Candida tropicalis* (MTCC 184), and *E. coli* (MTCC 448).[9,10] This study is also the continuation of the previous study. With no doubt, the next decades will focus on the research related to the antimicrobial agents present in the herbs, spices, wild plants, etc., for their antimicrobial properties. The isolation, purification, and study related to health- care applications should be undertaken in a guided manner and protocol established can pave path to tackle the challenges posed by new pandemics. Once the activity of the plant is identified, it should be followed by the identification of the potential phytochemical agents. The study carried out in the research can be further enhanced to make it more efficient in terms of solvent system used and the extraction strategies and methods. However, this study confirms the presence of antimicrobial agents in *T. vulgaris, R. officinalis* L., and *O. vulgare.*

CONCLUSION

From this study, it has been concluded that *T. vulgaris, R. officinalis* L., and *O. vulgare* are rich in phytochemicals with health-care applications. Further, studies need to be given emphasis for establishing knowledge on antimicrobial activities and other associated health-care applications. *T. vulgaris, R. officinalis* L., and *O. vulgare* have phytochemicals which are rich source of antioxidants, anti-inflammatory, skin care, hepatoprotective, and chemopreventive action. Thyme plant has a number of uses and has high economic importance. It has been used to cure diseases from thousands of years and thus has a wide application in field of medicine. Its different plant parts are used to treat different diseases. Similarly, *R. officinalis* L. has been used for its medicinal, tonic, astringent, diuretic, and diaphoretic properties in conventional and complementary alternative medicine. *O. vulgare* has been used in traditional medicines for such ailments as asthma, cramping, diarrhea, indigestion, and as an antiseptic. From the present research, it can be concluded that these plants can provide a solution to emerging pandemic if a detailed research is carried out. Study should focus on synergistic as well as individual plant applications; same is the case with the phytochemicals extracted from them. Furthermore, they can be studied for their interaction with antibiotics or/and their pharmacokinetic profile should be given high priority.

ACKNOWLEDGMENT

Authors are thankful to the IBST and SRMU for providing us this opportunity to carry out the work.

REFERENCES

1. Singh M, Vats S. Mathematically designed bioprocess for release of value added products with pharmaceutical applications from wastes generated from spices industries. Int J Pharm Sci Res 2019;10:130-8.
2. Vats S, Miglani K. Synergistic antimicrobial effect of cow urine and *Azadirachta indica* on infectious microbes. Int J Pharm Sci Res 2011;2:1781.
3. Bhargava P, Gupta N, Kumar R, Vats S. Plants and microbes: Bioresources for sustainable development and biocontrol. In: Plant Microbe Symbiosis. Cham: Springer; 2020. p. 153-76.
4. Vats S. Methods for extractions of value-added nutraceuticals from lignocellulosic wastes and their health application. In: Ingredients Extraction by Physicochemical Methods in Food. Cham: Springer; 2017. p. 1-64.
5. Ojha AK, Forster S, Kumar S, Vats S, Negi S, Fischer I. Synthesis of well-dispersed silver nanorods of different aspect ratios and their antimicrobial properties against gram positive and negative bacterial strains. J Nanobiotechnol 2013;11:42.
6. Li J, Tang M, Xue Y. Review of the effects of silver nanoparticle exposure on gut bacteria. J Appl Toxicol 2019;39:27-37.
7. Vats S, Maurya DP, Agarwal A, Shamoon M, Negi S. Development of a microbial consortium for the production of blend of enzymes for the hydrolysis of agricultural wastes into sugars. J Sci Ind Res 2013;72:585-790.
8. Vats S, Kumar R, Negi S. Natural food that meet antibiotics resistance challenge: *In vitro* synergistic antimicrobial activity of *Azadirachta indica*, *Terminalia chebula*, *Piper nigrum* and photoactivated cow urine. Asian J Pharm Biol Res 2012;2:122-6.
9. Singh RL, Gupta R, Dwivedi N. A review on anti-microbial activities of Triphala and its constituents. World J Pharm Pharm Sci 2016;5:535-58.
10. Vats S, Negi S. Use of artificial neural network (ANN) for the development of bioprocess using *Pinus roxburghii* fallen foliages for the release of polyphenols and reducing sugars. Bioresour Technol 2013;140:392-8.
11. Guiné RP, Matos S, Gonçalves FJ, Costa D, Mendes M. Evaluation of phenolic compounds and antioxidant activity of blueberries and modelization by artificial neural networks. Int J Fruit Sci 2018;18:199-214.
12. Sharma D, Javed S, Arshilekha, Saxena P, Babbar P, Shukla D, *et al*. Food additives and their effects: A mini review. Int J Curr Res 2018;10:69999-70002.
13. Rasool A, Bhat KM, Sheikh AA, Jan A, Hassan S. Medicinal plants: Role, distribution and future. J Pharmacogn Phytochem 2020;9:2111-4.
14. Bhuiyan FR, Howlader S, Raihan T, Hasan M. Plants metabolites: Possibility of natural therapeutics against the COVID-19 pandemic. Front Med 2020;7:444.
15. Erb M, Kliebenstein DJ. Plant secondary metabolites as defenses, regulators, and primary metabolites: The blurred functional trichotomy. Plant Physiol 2020;184:39-52.
16. Moeini A, Pedram P, Makvandi P, Malinconico M, D'Ayala GG. Wound healing and antimicrobial effect of active secondary metabolites in chitosan-based wound dressings: A review. Carbohydrate Polymers 2020;233:115839.
17. Andrade JM, Faustino C, Garcia C, Ladeiras D, Reis CP, Rijo P. *Rosmarinus officinalis* L.: An update review of its phytochemistry and biological activity. Future Sci OA 2018;4:FSO283.
18. Kompelly A, Kompelly S, Vasudha B, Narender B. *Rosmarinus officinalis* L.: An update review of its phytochemistry and biological activity. J Drug Deliv Ther 2019;9:323-30.
19. Bicchi C, Binello A, Rubiolo P. Determination of phenolic diterpene antioxidants in rosemary (*Rosmarinus officinalis* L.) with different methods of extraction and analysis. Phytochem Anal 2000;11:236-42.
20. Aouadi M, Sebai E, Saratsis A, Kantzoura V, Saratsi K, Msaada K, *et al*. Essential oil of *Rosmarinus officinalis* induces *in vitro* anthelmintic and anticoccidial effects against *Haemonchus contortus* and *Eimeria* spp. in small ruminants. Vet Med 2021;66:146-55.
21. Pesavento G, Calonico C, Bilia AR, Barnabei M, Calesini F, Addona R, *et al*. Antibacterial activity of oregano, rosmarinus and thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. Food Control 2015;54:188-99.
22. Al-Sereiti MR, Abu-Amer KM, Sena P. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J Exp Biol 1999;37:124-30.
23. Allegra A, Tonacci A, Pioggia G, Musolino C, Gangemi S. Anticancer activity of *Rosmarinus officinalis* L.: Mechanisms of action and therapeutic potentials. Nutrients 2020;12:1739.
24. Lorenzo-Leal AC, Palou E, López-Malo A, Bach H. Antimicrobial, cytotoxic, and anti-inflammatory activities of *Pimenta dioica* and *Rosmarinus officinalis* essential oils. BioMed Res Int 2019;2019:1639726.
25. Tural S, Turhan S. Antimicrobial and antioxidant properties of thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.) and laurel (*Lauris nobilis* L.)

essential oils and their mixtures. J Food 2017;42:588-96.
26. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Mar Contreras MD, Segura-Carretero A, *et al*. Thymol, thyme, and other plant sources: Health and potential uses. Phytother Res 2018;32:1688-706.
27. Pezzani R, Vitalini S, Iriti M. Bioactivities of *Origanum vulgare* L.: An update. Phytochem Rev 2017;16:1253-68.
28. Ličina BZ, Stefanović OD, Vasić SM, Radojević ID, Dekić MS, Čomić LR. Biological activities of the extracts from wild growing *Origanum vulgare* L. Food Control 2013;33:498-504.
29. Jan S, Rashid M, Abd-Allah EF, Ahmad P. Biological efficacy of essential oils and plant extracts of cultivated and wild ecotypes of *Origanum vulgare* L. BioMed Res Int 2020;2020:8751718.
30. World Health Organization. Laboratory Testing of 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases; 2020. Available from: https://www.apps.who.int/iris/handle/10665/330676. [Last accessed on 2020 Jan 17].
31. Gorbalenya AE, Baker SC, Baric R, Groot RJ, Drosten C, Gulyaeva AA, *et al*. Severe acute respiratory syndrome-related coronavirus: The species and its viruses-a statement of the Coronavirus Study Group. Nat Microbiol 2020;5:536-44.
32. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah N, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antivir Res 2020;176:104742.
33. Kannan S, Ali PS, Sheeza A, Hemalatha K. COVID-19 (novel coronavirus 2019)-recent trends. Eur Rev Med Pharmacol Sci 2020;24:2006-11.
34. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol 2020;94:e00127-20.
35. Shariatzadeh SM, Miri SA, Cheraghi E. The protective effect of Kombucha against silver nanoparticles-induced toxicity on testicular tissue in NMRI mice. Andrologia 2021;2021:e13982.
36. Kaur A, Vats S, Rekhi S, Bhardwaj A, Goel J, Tanwar RS, *et al*. Physico-chemical analysis of the industrial effluents and their impact on the soil microflora. Proc Environ Sci 2010;2:595-9.
37. Aniyikaiye TE, Oluseyi T, Odiyo JO, Edokpayi JN. Physico-chemical analysis of wastewater discharge from selected paint industries in Lagos, Nigeria. Int J Environ Res Public Health 2019;16:1235.
38. Aashigari S, Goud R, Sneha S, Vykuntam U, Potnuri NR. Stability studies of pharmaceutical products. World J Pharm Res 2018;8:479-92.
39. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005;100:80-4.
40. Sytar O, Brestic M, Hajihashemi S, Skalicky M, Kubeš J, Lamilla-Tamayo L, *et al*. COVID-19 prophylaxis efforts based on natural antiviral plant extracts and their compounds. Molecules 2021;26:727.
41. Vats S, Kumar R, Miglani AK. Isolation, characterization and identification of high salinity tolerant, heavy metal contaminant and antibiotics resistant amylolytic-thermophilic *Pseudomonas* Sp. Int J Pharm Sci Rev Res 2011;10:125-9.
42. Budhiraja N, Srivastava P, Agrahari S, Shukla D, Mudgil B, Saxena S, *et al*. Plant-microbe-metal interactions: A biochemical and molecular analysis for phytoremediation. In: Plant Microbiome Paradigm. Cham: Springer; 2020. p. 71-92.
43. Bozin B, Dukic-Mimica N, Simin N, Anackov G. Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem 2006;54:1822-8.
44. Schelz Z, Molnar J, Hohmann J. Antimicrobial and antiplasmid activities of essential oils. Fitoterapia 2006;77:279-85.
45. Sahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, *et al*. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control 2004;15:549-57.
46. Sienkiewicz M, Lysakowska M, Pastuszka M, Bienias W, Kowalczyk E. The potential of use basil and rosemary essential oils as effective antibacterial agents. Molecules 2013;18:9334-51.

Source of Support: Nil. **Conflicts of Interest:** None declared.