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Original article

Studies on the isolation and identification of the antibacterial compound from *Prunella vulgaris* L. Flower extract

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ABSTRACT

Prunella vulgaris is an important medicinal plant with a wide range of pharmacological properties. The present research is aimed to investigate the functional groups, phytochemical constituents, *in-vitro* antibacterial activity and isolation of antibacterial compound from flower extract of *P. vulgaris*. Here, we examined the antibacterial activity of *P. vulgaris* extract against gram positive (*Streptococcus pneumonia*, *Enterococcus faecalis* and *Staphylococcus aureus*) and gram negative (*Escherichia coli and Klebsiella pneumonia*) bacterial strains. Based on our results, the extract showed strong antibacterial activity against all of the pathogens used in this study. The minimum inhibitory concentration (MIC) of *P. vulgaris* extract was determined by broth micro dilution method. Further, the extract was subjected to column chromatography to get the active fractions. To identify and isolate the antibacterial molecule, the active fraction (fraction-6) was subjected to spectral analysis using the Fourier-transform infrared spectroscopy (FT-IR), ^{1}H ^{13}C nuclear magnetic resonance (NMR), and mass spectroscopy (MS). On the basis of functional group analysis, molecular formula and molecular mass, the isolated compound was identified as [(**2**-(E)-3-(3-4-dihydroxyphenyl) acryloyloxy)-3-(3, dihydroxyphenyl) propanoic acid (Rosmarinic acid)] from the active fraction of *P. vulgaris* flower extract. In conclusion *P. vulgaris* has enormous therapeutic and pharmacological significance and can be used in the research and development of antibacterial medications as well as other pharmacological endeavors.

1. Introduction

Plants have been an incredible source of therapeutic compounds, and their medical efficacies are still being studied on a global scale. The majority of pathogenic bacteria have developed resistance to antibiotics as a result of their excessive use, incorrect prescription, and constant mutation which poses a substantial threat to public health. An increasing trend of resistant antimicrobial compounds is spreading at surprising rate and infections are turning out to be more stern and brutal, hence transmitted easily (Wang et al. (2020). Reports from WHO suggest that in forth coming years many infectious diseases are turning out to be incurable and uncontainable (Wilder-Smith et al. (2020). AMR infections are thought to be the cause of about 700,000 annual deaths, and by the year 2050, that number is expected to rise to 10 million (O'Neill, 2016). Some of the AMR strains such as *S. aureus, S. pneumonia, P. aeruginosa*, and *Mycobacterium tuberculosis* pose a major threat to human health as they exhibit resistance to many antibiotic groups (Lowy, 2003; Boucher et al., 2009). Additionally, severe side effects of artificial drugs have led to the use of potential alternative medications among natural sources (Anand et al. (2019). Herbal medicine has proven to be suitable substitutes for these synthetic drugs with negligible side effects (Srivastava et al. (2019). Due to the broad diversity of secondary metabolites (polyphenols, alkaloids, tannins, etc.) found in medicinal plants, crude extracts may be used as an alternative for combating antibiotic resistance.

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Scientists all over the world have gained potential interest in investigating the antibacterial activities of plant products along with their mechanism of action and possible utilization and concentration (Jain et al. (2019). The extracts from herbal products represent a great deal of chemical multiplicity, might potentially could become replacements against MDR (multidrug resistant) infections (Vaou et al. (2021).

The ethnobotanical investigations have been found to be effective in revealing the potential of medicinal herbs and paved way for isolating active components from traditional medicinal plants located in climates of arid to alpine regions (Jalli et al., 2015). The family of lamiaceae has a broad-based distribution (Li et al., 2016).The inflated Lamiaceae family contain nearly 236 genera and 7100 species (Wang and Wang, 2011). In India about 64 genera, 380 species of plants related to Lamiaceae family has been recognized so far, however in Uttarakhand alone, about 33 genera and 71 species are currently documented (Bisht et al. (2012). The parts of these plants are commonly full of fragrance and comprise many extensively used cookery herbs (Rama Rao et al. (2015). Pharmaceutical potential of Lamiaceae species are primarily due to the composition of soaring amount of impulsive components (Mohanty et al. (2022). One of the most important member of Lamiaceae family is *P. vulgaris* which is also called "all-heal and self-heal" owing to its incredible medicinal properties. Previous studies have reveled that *P. vulgaris* has a wide range of medicinal potentials, including anti-cancerous, antiseptic, anti-rheumatic, anti-spasmodic, and anti-HSV properties (Chen et al., 2009; Fazal et al., 2020). The present research is aimed to investigate the functional group analysis, phytochemical analysis, *in-vitro* antibacterial activity and isolation of antibacterial compound from flower extract of *P. vulgaris*.

2. Materials and methods

2.1. Collection and preparation of plant material

The *P. vulgaris* plant was collected from Aharbal vicinity of Jammu and Kashmir India. *P. vulgaris* was identified and authenticated by Department of Horticulture, faculty of Agriculture Annamalai University (India) with specimen no. Hort./Id.2/2020. One voucher specimen was kept at Department of Horticulture Annamalai University. The dried plant material was ground by the electric grinder. The ground powder obtained was extracted with methanol and petroleum ether solvents to yield methanol flower extract (MEFE) and petroleum ether flower extract (PEFE) by using soxhlet extractor.

2.2. Preliminary phytochemical screening

The range of phytochemical and bioactive constituents in the methanol and petroleum ether extracts were analysed by the methodology of Goyal et al., 2010.

2.3. Bacterial strains used for examining antibacterial activity of P. Vulgaris

To examine the antibacterial properties of *P. vulgaris*, three gram positive bacterial species *viz., S. pneumonia, E. faecalis* and *S. aureus* and two gram negative bacterial species *K. pneumonia* and *E. coli* were selected and tested respectively. Pure bacterial cultures were obtained from Department of Microbiology Annamalai University Chidambaram India and were maintained in the laboratory by usual sub-culturing on to the nutrient agar (NA) incubated at 37 °C.

2.4. Screening of antibacterial activity

The minimum inhibitory concentration (MIC) of the methanol flower extract (MEFE) along with the isolated compound was estimated by dilution method as reported by (Prakash et al. (2010).

2.5. Column chromatography

In this study the compound from *P. vulgaris* flower extract was isolated by using column chromatography. The crude MEFE obtained after soxhlet extraction was subjected to column chromatography. The bioactive secondary metabolites were separated chromatographically with a silica gel column packed with normal phase by the method followed by (Hwang et al. (2013). The preparation of packed silica column was done by using 12.5 g of silica gel. The dried MEFE was solubilised in DMSO. Then, 0.5 g of silica was added, and allowed to dry so as to make a dry band, and then added to the top of the packed silica column. A total of five fractions were collected, each fraction containing 10 ml. The resemblance of the fractions collected was checked by using thin-layer chromatography (TLC) plates with ethyl acetate and hexane in the ratio of 4: 6. The collected fractions were screened for antimicrobial activity using the well diffusion method. The column was eluted with solvents of increasing polarities from hexane to methanol and 12 fractions were collected. The F6 fraction (chloroform: ethanol combination, 9.0:1.0) was subsequently subjected to separation and purification of active chemical based on its greatest antibacterial activity.

2.6. Infrared spectral analysis

The Fourier-transform infrared spectroscopy (FT-IR) is a technique to provide a plot of absorption of infrared light by the sample as a function of frequency or wave length. It is perhaps the most dominant tool to identify different types of functional groups (chemical bonds). Thus, to interpret the infrared absorption spectrum, the functional groups in a molecule can be estimated. FT-IR spectra was obtained by Spectrum RX1 FT-IR spectrophotometer (Perkin Elmer, USA) operational in 4000–400 cm^{-1} region of wave length; fitted with potassium bromide (KBr) beam splitter, DTGS detector and nichrome source. Totally 100 scans were obtained for final spectrum with 4 cm^{-1} resolution. The powdered and dried extract was encapsulated in KBr pellet for the making translucent sample discs.

2.7. NMR spectral analysis

The proton and proton decoupled ^{13}C NMR spectra at 400.13 and 100.62 MHz respectively were recorded at room temperature by using a Bruker 400.13 MHz NMR spectrometer (Brukerbiospin, California, USA). TMS (tetramethylsilane) was used as an internal standard for both spectra.

2.8. Mass spectral (MS) analysis

MS was done by using Shimadzu Co. ltd (Kyoto, Japan) in both positive and negative ion modes. The parameters were as follows: negative and positive ionization modes scan range from *m*/*z* 100 to 1000, DL temperature 250 °C.

3. Results

3.1. Preliminary phytochemical screening of P. Vulgaris flower extract

The preliminary phytochemical evaluation conducted on flower extracts of *P. vulgaris* revealed the presence of many bioactive con-

stituents such as carbohydrates, tannins, reducing sugars, saponins, flavonoids, steroids, alkaloids, anthroquinone, glycosides, terpenoids, coumarins and phenolic (Table. 1).

3.2. Assessment of minimum inhibitory concentration (MIC)

The MIC values of *P. vulgaris* flower extracts, ciprofloxacin is represented in Table 3**.** MIC values varied from 100 to 400 μg/ml for flower methanol extracts (MEFE) and 100 to 500 μg/ml for petroleum ether extracts (PEFE). Flower methanolic extracts of *P. vulgaris* showed better MIC (>100 μg/ml) against *E. coli* while as petroleum ether extracts revealed better results of MIC against *S. aureus* (100 μg/ml).

3.3. Characterization of isolated compound

In the current investigation, a total of twelve fractions were produced using column chromatography, with fraction 6 (F6) displaying the highest antibacterial activity. Further, the F6 fraction was subjected to spectral analysis for identification of active compound respectively.

Table 1
Preliminary phytochemical screening of flower of *P. vulgaris.*

Chemical components	MEFE	PEFE
Carbohydrates	++	++
Reducing sugars	–	++
Tannins	–	++
Saponins	++	–
Flavonoid	++	–
Steroids	–	++
Alkaloids	++	+
Anthraquinone	++	++
Glycosides	+	–
Terpenoids	+	++
Coumarins	+	–
Phenolics	++	++

3.3.1. FT-IR spectrum of 2-(E)-3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3,dihydroxyphenyl) propanoic acid (Rosmarinic acid).

FT-IR analysis is a useful analytical method for identifying the functional groups present in biological samples. In this study, the FT-IR spectrum of antibacterial compound 2- (E) – 3-(3-4-dihydroxyphenyl) acryloyloxy)-3-(3, 4- dihydroxyphenyl) propanoic acid (Rosmarinic acid) was analysed in the range of 4000–500 cm^{-1}. The $V_{C=N}$ band was observed at 1734 cm^{-1}, C = 0 was given at 1598, V_{N-H} band was found at 3448 cm^{-1} (Fig. 1).

3.3.2. ^{1}H and ^{13}C NMR spectral analysis of 2- (E) – 3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3,dihydroxyphenyl) propanoic acid (Rosmarinic acid)

3.3.2.1. ^{1}H NMR (400.13 MHz, CDCl) δ ppm. In this study NMR profiling ^{1}H NMR (400.13MHZ, δ, ppm): 9.65 [S, 1H, H(6)], 6.57–7.14 [(m,8H) (Ar-H], 3.56 [(d,1H,11 (H)], 2.73 [(t,1H, 10 (H)] was carried out.^{1}H NMR spectrum of the obtained compound are shown in figure 4.3.9.65 ppm represents singlet peak and the aromatic protons are found to be resonating in between 6.57 and 7.14 ppm. The resonance in up field region at 3.56 ppm represents at doublets. The triplet peak is observed at 2.73 (Fig. 2).

3.3.2.2. ^{13}C NMR (100.62 MHz, CD Cl) δ ppm. ^{13}C NMR analysis of the isolated compound has been revealed as (100.62 MHZ, CDCL3-d6, δ, ppm): 174.49 [(C = 0, C (18)], 161.34 [(C = 0, (9)], 126.52–139.35 (Ar-C)], 79.68 (C-18), 39.30 (C-10), 139.38–143.19 (ipso-carbons).^{13}C NMR is depicted in Fig. 2. In ^{13}C NMR spectrum carbonyl carbons vibrate at 174.49 ppm and 161.34 ppm respectively. The ipso carbons are the phenyl rings and vibrate at 139.38 – 143.19 ppm. The signal generated by aromatic carbons appeared between 126.52 and 139.35 ppm. The signals at 39.30 ppm and 79.68 ppm are assigned to C-18 and C-18 respectively (Fig. 2).

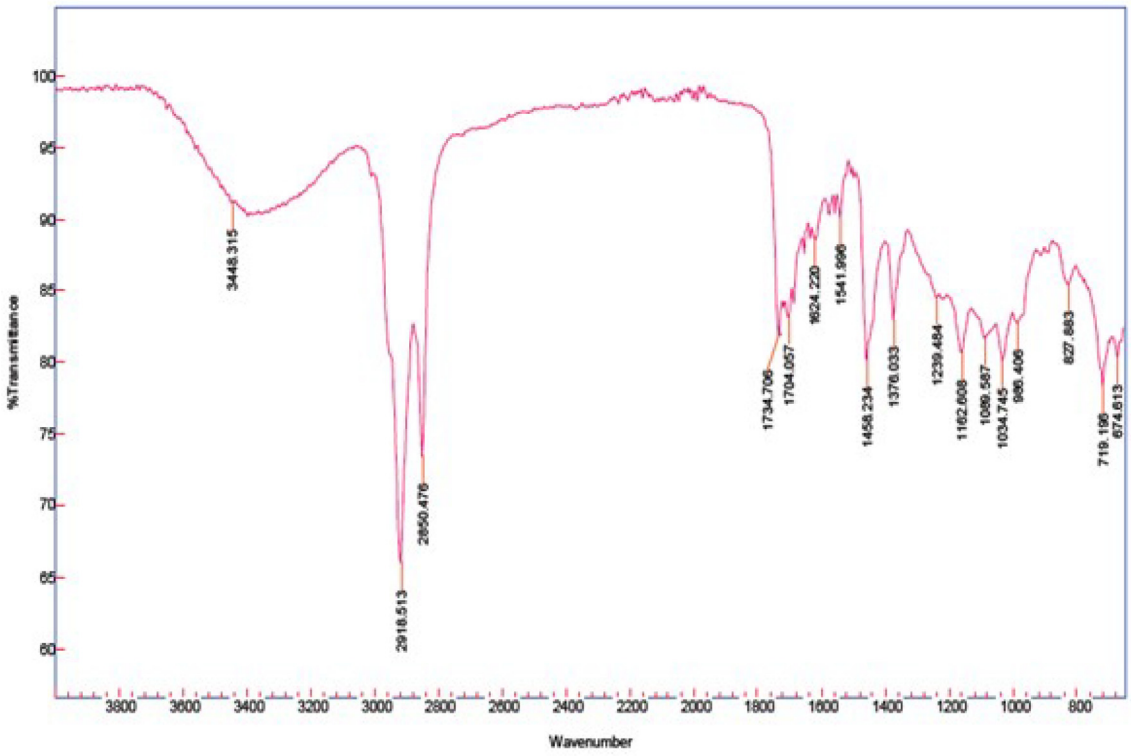

Fig. 1. Showing FT-IR spectrum of 2- (E) –3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3, 4- dihydroxyphenyl) propanoic acid (Rosmarinic acid).

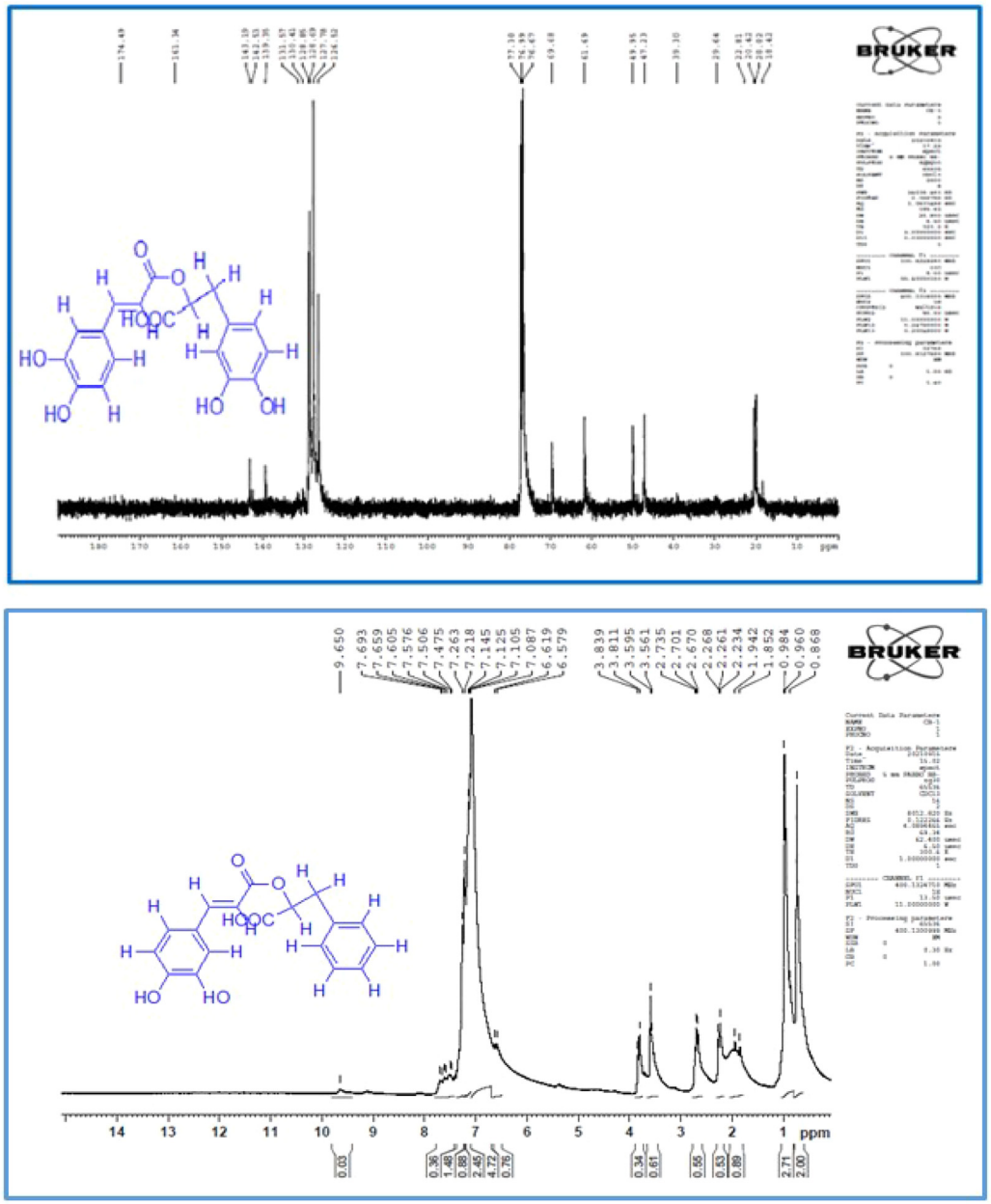

Fig. 2. ^{1}H and ^{13}C NMR of the isolated compound.

The molecular formula for the compound was predicted to be $C_{18}H_{16}O_8$.

3.3.3. MS analysis of 2- (E)- 3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3, 4- dihydroxyphenyl) propanoic acid (Rosmarinic acid)

The compound isolated was obtained as the liquid and the MS spectrum was recorded as mass of $[m/z]^{\pm 1}$ = 360.20. On the basis of the spectral observations, the molecule isolated was identified as 2- (E) – 3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3, 4- dihydroxyphenyl) propanoic acid (Rosmarinic acid) (Fig. 3).

4. Discussion

The threat of antibiotic resistance to human health and to the medical treatment of disease is growing on a worldwide scale. The scientific community continues to face challenges as a result of the current issue of emerging new antibiotic resistant pathogenic strains (Breijyeh et al. (2020). On the other hand side effects of antibiotics have increased drastically along with their insufficient supply and increasing cost (Sulis et al. (2021). Therefore, the immediate demand is to uncover a way out to such a crisis. Presently, the best therapeutic treatments for managing human health-related illnesses and infections continue to be derived from medicinal plants and their compounds. The current investigation has been carried out to determine the antimicrobial potential of the *P. vulgaris* flower extract and to identify the compound with anti microbial potential. The plant has been recognized to produce a significant quantity of secondary bioactive constituents in the form of phenolics, flavonoids, tannins, and other useful alkaloids. *P. vulgaris* has shown a good content of essential oils with antimicrobial and antioxidant activities (Patel et al. (2021).

For investigating the bioactive compounds present in *P. vulgaris* flower, the organic solvents methanol and petroleum ether were used for extraction analysis. The methanolic extract of *P. vulgaris* flower has yielded better results for phytochemical analysis than

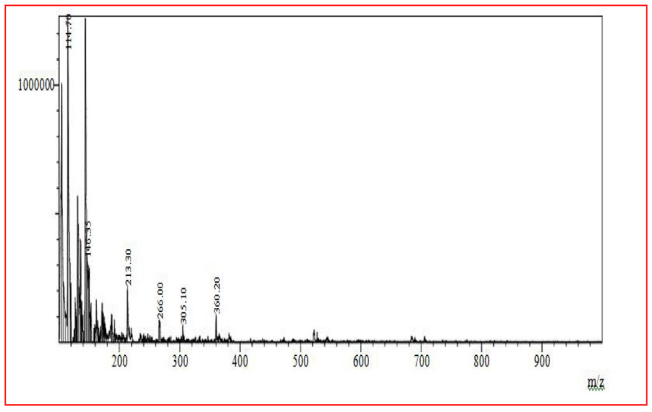

Fig. 3. MS of2- (E) −3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3, 4- dihydroxyphenyl) propanoic acid (Rosmarinic acid).

Table 2
MIC values of MEFE and PEFE of *P. vulgaris* against different bacterial pathogens.

Bacterial strains	MIC (µg/mL)		
	Petroleum ether	Methanol	Ciprofloxacin
Escherichia coli	>200	>100	0.718
E. faecalis	>150	>150	1.19
K. Pneumoniae	>500	>300	0.15
S. pneumonia	200	>400	0.567
S. aureus	100	100	1.14

the extracts of petroleum ether. Antimicrobial activities of both the solvent extracts were screened against three gram positive and two gram negative bacterial strains. The methanolic flower extract of *P. vulgaris* verified noteworthy inhibition zones in case of all the strains tested at different concentrations than the results obtained from petroleum ether extracts, the maximum zones of inhibition were recorded at a concentration of 150 µg/ml. Minimum inhibitory concentration assessment of *P. vulgaris* flower extract was also done in which methanol extracts revealed better results than petroleum ether extracts (Table 2). Previous studies have also revealed the potential antibacterial activity of *P. vulgaris* extracts against different bacterial pathogens (Salem et al., 2021). The results obtained from initial screening against all of the tested bacterial strains, the flower crude extract of *P. vulgaris* was subjected for column chromatography to get the active fractions. Totally twelve fractions were obtained with F6 (fraction 6) showing maximum antibacterial activity.

Spectral analysis results revealed that the bioactive compound present was 2- (E) – 3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3, 4- dihydroxyphenyl) propanoic acid (Rosmarinic acid). Different studies on rosmarinic acid from *P. vulgaris* with several origins have been reported previously (Chua et al. 2018; Trócsányi et al., 2020; Fatemi et al. 2019). Rosmarinic acid has shown antioxidant activity in hepatic stellate cells (HSCs) by activating GSH synthesis and participating in NF-B-dependent inhibition of MMP-2 action. Rosmarinic acid stabilizes lipid peroxidation process, generation of reactive oxygen species, production of peroxynitrite, complement factor and pro-inflammatory mediators like cytokines and chemokines. It can be a huge breakthrough molecule for the treatment of depression and in inducing variations in BDNF levels and in ERK11/2 signalling in pharmacological science· Studies suggested that rosmarinic acid can act through a variety of mechanisms, thus exerting anti-inflammatory, antioxidant, anti-proliferative and also promote cell apoptosis (Nadeem et al. (2019). In the present study the existence of rosmarinic acid in *P. vulgaris* flower extract has been observed to induce antibacterial activity after screening on several human pathogens.

5. Conclusions

The compound, 2- (E) – 3-(3–4-dihydroxyphenyl) acryloyloxy)-3-(3, 4- dihydroxyphenyl) propanoic acid (Rosmarinic acid) was isolated from the methanolic extracts of flower of *P. vulgaris.* The compound was found to possess effective antibacterial potential as revealed from previous studies. However, the possible mechanism of action of the isolated compound against different bacterial species, impact on the health of humans needs to be revealed. The findings in our study suggested that the bioactive properties of the plant extracts provide comprehensive proof for the use of this plant in clinical trials. Rosmarinic acid also showed inhibitory activities against *E. coli* and *S. carnosus* through diminishing cell count and cell amount. On the basis of these results, the bioactive compound in the form of Rosmarinic acid derived from *P. vulgaris* flower could be explored as a source of antibacterial agent, and may help to supplement the demand of synthetic antibiotics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anand, U., Jacobo-Herrera, N., Altemimi, A., Lakhssassi, N., 2019. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites 9 (11), 258.
Bisht, V.K., Rana, C.S., Negi, J.S., Bh, A.K., Purohit, V., Kuniyal, C.P., Sundriyal, R.C., 2012. Lamiaceous ethno-medico-botanicals in Uttarakhand Himalaya, India. J. Med. Plants Res. 6 (26), 4281–4291.
Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E., Gilbert, D., Rice, L.B., et al., 2009. Bad Bugs, No Drugs: No ESKAPE! an update from the infectious diseases society of america. Clin. Infect. Dis. 48, 1–12.
Breijyeh, Z., Jubeh, B., Karaman, R., 2020. Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 25 (6), 1340.
Chen, C.Y., Wu, G., Zhang, M.Z., 2009. The effects and mechanism of action of Prunella vulgaris L. extract on Jurkat human T lymphoma cell proliferation. Chin Ger. J. Clin. Oncol. 8 (7), 426–429.
Chua, L.S., Lau, C.H., Chew, C.Y., Ismail, N.I.M., Soontorngun, N., 2018. Phytochemical profile of Orthosiphon aristatus extracts after storage: Rosmarinic acid and other caffeic acid derivatives. Phytomedicine 39, 49–55.
Fatemi, F., Abdollahi, M.R., Mirzaie-asl, A., Dastan, D., Garagounis, C., Papadopoulou, K., 2019. Identification and expression profiling of rosmarinic acid biosynthetic genes from Saturejakhuzistanica under carbon nanotubes and methyl jasmonate elicitation. Plant Cell, Tissue and Organ Culture (PCTOC) 136 (3), 561–573.
Fazal, H., Abbasi, B.H., Ahmad, N., Noureen, B., Shah, J., Ma, D., Chuanliang, L., Akbar, F., Uddin, M.N., Khan, H., Ali, M., 2020 Dec. Biosynthesis of antioxidative enzymes and polyphenolics content in calli cultures of Prunella vulgaris L. in response to auxins and cytokinins. Artif. Cells Nanomed Biotechnol. 48 (1), 893–902.
Goyal, A.K., Middha, S.K., Sen, A., 2010. Evaluation of the DPPH radical scavenging activity, total phenols and antioxidant activities in Indian wild Bambusa vulgaris“ Vittata” methanolic leaf extract. J. Nat. Pharmaceut. 1 (1).
Hwang, Y.J., Lee, E.J., Kim, H.R., Hwang, K.A., 2013. In vitro antioxidant and anticancer effects of solvent fractions from Prunella vulgaris var. lilacina. BMC complement. Alternat. Med. 13 (1), 1–9.
Jain, C., Khatana, S., Vijayvergia, R., 2019. Bioactivity of secondary metabolites of various plants: a review. Int. J. Pharmaceut. Sci. Res. 10 (2), 494–498.
Jalli, R., Aravind, J., Pandey, A., 2015. Conservation and management of endemic and threatened plant species in India: An overview. Plant Biol. Biotechnol., 461–486
Li, B., Cantino, P.D., Olmstead, R.G., Bramley, G.L., Xiang, C.L., Ma, Z.H., Zhang, D.X., 2016. A large-scale chloroplast phylogeny of the Lamiaceae sheds new light on its subfamilial classification. Sci. Rep. 6 (1), 1–18.

Lowy, F.D., 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. J. Clin. Investig. 111, 1265–1273.
Mohanty, S., Dash, K.T., Jena, S., Ray, A., Sahoo, A., Kar, S.K., Panda, P.C., 2022. Chemical Composition, Antimicrobial and Cytotoxic Activity of the Essential Oil of Platostomahispidum, an Unexplored Species of Lamiaceae. J. Essent. Oil Bear. Plants, 1–11.
Nadeem, M., Imran, M., Aslam Gondal, T., Imran, A., Shahbaz, M., Muhammad Amir, R., Martins, N., 2019. Therapeutic potential of rosmarinic acid: A comprehensive review. Appl. Sci. 9 (15), 3139.
O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance. http://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf Date: May, 2016 (accessed Aug 14, 2022).
Patel, J.K., Joshi, C.K., Sharma, M.K., 2021. Prunella vulgaris L Potentiates Answer to the Emergence of Dreaded Antibiotic Resistance. J. Pure Appl. Microbiol. 15 (3), 1429–1442.
Prakash, O., Rajput, M., Kumar, M., Pant, A.K., 2010. Chemical Composition and antibacterial activity of Rhizome Oils from Hedychiumcoronarium Koenig and Hedychium spicatum Buch-Ham. J. Essent. Oil Bear. Plants. 13 (2), 250–259.
Rama Rao, V., Shiddamallayya, N., Kavya, N., Kavya, B., Venkateshwarlu, G., 2015. Diversity and therapeutic potentiality of the family Lamiaceae in Karnataka State, India: an overview. History 13 (37), 6–14.
Salem, M.S.E., Mahfouz, A.Y., Fathy, R.M., 2021. The antibacterial and antihemolytic activities assessment of zinc oxide nanoparticles synthesized using plant extracts and gamma irradiation against the uro-pathogenic multidrug resistant Proteus vulgaris. Biometals 34, 175–196.
Srivastava, A., Srivastava, P., Pandey, A., Khanna, V.K., Pant, A.B., 2019. Phytomedicine: A potential alternative medicine in controlling neurological disorders. In New Look to Phytomedicine, 625–655.
Sulis, G., Sayood, S., Gandra, S., 2021. Antimicrobial resistance in low-and middle-income countries: current status and future directions. Expert Rev anti-infective Ther., 1–14
Trócsányi, E., György, Z., Zámboriné-Németh, É., 2020. New insights into rosmarinic acid biosynthesis based on molecular studies. Curr. Plant Biol. 100162.
Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., Bezirtzoglou, E., 2021. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms 9 (10), 2041.
Wang, C.H., Hsieh, Y.H., Powers, Z.M., Kao, C.Y., 2020. Defeating antibiotic-resistant bacteria: exploring alternative therapies for a post-antibiotic era. Int. J. Mol. Sci. 21 (3), 1061.
Wang, Y.C., Wang, Y.R., 2011. Levels of phenolic acids and tanshinones in Salvia miltiorrhizaBge. cv. sativa and Salvia bowleyanae Dunn roots during growth. Journal of Biologically Active Products from. Nature 1 (1), 7–18.
Wilder-Smith, A., Chiew, C.J., Lee, V.J., 2020. Can we contain the COVID-19 outbreak with the same measures as for SARS? Lancet. Infect. Dis 20 (5), 102–107.