SMTCT
Society of Land Measurements and Cadastre from Transylvania

Kumari SP and Meiyappan E (2024)
Notulae Scientia Biologicae
Volume 16, Issue 1, Article number 11758
DOI:10.15835/nsb16111758
Research Article

NSB
Notulae Scientia Biologicae

Potential of diallyl sulfide from bulbs of *Allium parvum* to inhibit the growth and biofilm formation in *Malassezia furfur* MTCC 1374

Saravana P. KUMARI*, E. MEIYAPPAN

*RVS College of Arts and Science, Department of Microbiology, Coimbatore, India; sarankumaribs@gmail.com (*corresponding author); researchinmicro@gmail.com*

Abstract

The bulbs of *Allium parvum* are used as a flavoring agent in diet and are conventionally assumed to treat skin infections. The current study evaluates the phytochemicals from organic extract of *A. parvum* and their antifungal activity against biofilm-forming dermatophytic fungi *Malassezia furfur* MTCC 1374, yeast found in the normal flora of the skin, causes superficial infections, systemic infections like pulmonary infections, fungemia, etc., and implant-associated infection by biofilm formation. Biofilm formation enables the fungi to escape from antifungal agents and renders resistance to antifungal agents. The phytochemical assay for the organic extract of the onion bulb documented that *A. parvum* contains phenol (58 + 0.1 mg GAE/g), flavonoids (112 + 0.12 mg QE/g), saponins (1.45 + 0.1 mg AE/g), and tannins (4.1 + 0.03 g TA/g). The FT-IR and GC-MS analysis revealed the presence of specific compounds, alkaloids such as glycosides and quinolones, plant flavonoids such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-ester, phenolics, saponins such as butanoic acid and gluconic acid, and organic sulfur compounds such as diallyl sulfide, thiopyran tetrahydro-ester, allyl methyl trisulfide, and allyl methyl di sulfide. These compounds inhibited the growth of *M. furfur* at a minimum inhibitory concentration (MIC) value of 6.25 mg/ml and its biofilm at a minimum biofilm inhibitory concentration (MBIC) value of 12.5 mg/ml. Further studies required to explore the possible mechanism in controlling the biofilm, so, as to recommend as fungal control agent in medical sector.

Keywords: *Allium parvum*; bioautography; *Malassezia furfur*; minimum biofilm inhibitory assay; minimum inhibitory concentration

Introduction

Onion bulbs of *Allium parvum* are well-known as nutraceuticals and used as a flavoring agent in food worldwide. For its characteristic flavor used as the third most essential horticultural spice with a substantial commercial value. Apart from its culinary virtues, *A. parvum* is traditionally used for its medicinal properties in a plethora of indigenous cultures. Chemically the onion bulb contains 89% water, 1.5% protein, and vitamins B1, B2, and C, along with potassium and selenium. Studies proposed that dietary intake improves digestion, mental health and lowers down toxigenicity of oils (Upadhyay, 2016). *A. parvum* was found to possess a

Received: 17 Oct 2023. Received in revised form: 29 Nov 2023. Accepted: 18 Mar 2024. Published online: 29 Mar 2028.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

panoply of bioactive compounds with numerous pharmacological properties, including antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-diabetic, hypolipidemic, anti-hypertensive, anticancer, and immunoprotective effects. Although a large number of *in vitro* and *in vivo* studies have been conducted, several limitations and research gaps have been identified which need to be addressed to commercialize the phytochemical as medicine (Teshika *et al*., 2019).

A major constraint in the commercialization of drugs from onions is the separation of phytochemicals and standardization of extraction methods with polar or non-polar solvents, primarily aimed at extracting rapidly without any bioreactivity, alteration in phytopharmaceuticals, solvent toxicity, or interference with further bioassay (Velavan, 2015). Separated phytochemical compounds are characterized for identifying the bioactive principle in the herbs. Both conventional techniques include qualitative biochemical assays and nonconventional techniques like GC, HPLC, LC, NMR, UPLC, GC-MS, HPLC-MS, HPLC-SPE-NMR, HPTLC-MS, UP-HPLC, and UPLC-DAD-TOF-MS are recommended for the analysis (Yadav and Agarwal, 2011). Further quantitative analysis of the phytochemicals in the herb is more comprehensive and useful for drug dosage analysis, standardization, explanation of the medicinal potentials of plants, and determination of the toxicity levels of plants. In pharmacognosy, the newly identified and purified compounds from edible plant sources are more in demand for medicinal purposes, as they do not have any side effects, are available in ample quantities, easy to harness, and are readily acceptable as derived from edible plant parts.

Previous studies on bulbs of *A. parvum* red and white varieties showed chemotypic variations and displayed strong antimicrobial and antioxidant activities. Essential oil from the onion bulb showed antimicrobial activity and inhibited growth of the fungi *Aspergillus versicolor* and *A. flavum,* and sterigmatocystin production. Phenolics and flavonoid compounds from the yellow onion, reported to protect from oxidative damage and antioxidant response. Flavonoids from onion showed blood-brain barrier permeation and neuroprotective effects. Antifungal saponins isolated from bulbs of white onion, *A. parvum,* showed strong antifungal activity (Mnayer *et al.,* 2014).

In the current scenario, a widespread fungal infection that invariably affects all ages and gender is dandruff caused by *Malassezia furfur*. *M. furfur* is a lipid-dependent, biofilm-forming, monophyletic genus commensal of human scalp, that occupies 80% of human skin and correlates with several common skin problems. It is implicated in several common dermatologic disorders, including seborrheic dermatitis (SD), pityriasis versicolor (PV), and Malassezia folliculitis, and systemic infections such as peritonitis, fungemia, pulmonary infection, and catheter-acquired sepsis in patients receiving lipid parenteral nutrition (Kaneko *et al.,* 2012; Iatta *et al.,* 2014). Immunocompromised patients have the highest systemic and implant-associated infections (33%). SD is common in the winter months and slightly predominant in males, especially above 50 years of age (Vest and Krauland, 2022). The treatment for fungal infection primarily aim to reduce *Malassezia* sp., proliferation, and to reduce the resultant inflammatory response. First-line therapy includes topical application of fungistatic and fungicidal compounds or corticosteroids, anti-inflammatory, and antifungal chemicals like 1% zinc pyrithione, selenium sulfide, ciclopirox olamine, or 2% of ketoconazole. These assured topical drugs are not permitted for systemic antifungal therapy or to treat the resistant biofilm-forming strains (Roques *et al.,* 2006). Moreover, repeated usage of antifungal drugs makes the organism evolve as a resistant form or adapt to develop biofilm, which protects it from the inhibitory of the medicine. In serious conditions, biofilm formation encounter in invasive systemic infections by *M. folliculitis* warrant immediate removal of catheters.

Recent studies have demonstrated that *Malassezia* sp., susceptible to the drugs triazoles and amphotericin B, evolved as resistant form later and reported reoccurrences of the same infection in many patients (Drake *et al*., 1996; Tragiannidis *et al*., 2010; Borda *et al*., 2015). Several research articles have been published in an endeavor to validate such traditional claims. Nonetheless, and its mechanism specific compound is still a dearth. Meanwhile, an update on detailed compilation and critical analysis of the traditional

and ethnopharmacological propensities of *A. parvum* is required. As a result, the current study aims to analyze the phytochemical composition and elucidate its pharmacological properties systematically.

Materials and Methods

Collection of Malassezia furfur MTCC 1374

The standard strain of *Malassezia furfur* MTCC 1374 was procured from the Microbial Culture Collection Centre of IMTECH, Chandigarh. The culture was rehydrated with warm Potato dextrose broth for 6 hours, and sub-cultured on Potato Dextrose Agar medium with 2% of olive oil. The inoculated plates were incubated at 30 °C for 3 days for established growth and pigment production. Colonies were stained with Lactophenol cotton blue and evaluated for the typical colony morphology on Sabouraud Dextrose agar (SDA) supplemented with 2% olive oil.

Collection of onion bulb and organic solvent extraction

Onion bulbs of *Allium parvum* were collected from farm fields at Selakaraichal (N 10° 53.9718' E 77° 11.0308'), Coimbatore, Tamilnadu, India and collected plant samples were validated at the Botanical Survey of India, Southern Circle, Coimbatore, India. The outer scales of onion bulbs were removed, freeze-dried at – 20 °C for 48hrs and ground into a fine mixture. Then 10 g of the ground plant sample was soaked in 10 ml of distilled water/ absolute methanol/ ethanol/ chloroform/ acetone for 30 min by shaking in an incubator at 37 °C. After passing through Whatman No. 1 filter paper, each suspension was collected in a different test tube. An equal volume of ethyl acetate was added to the filtrate, incubated for 30 minutes, and the ethyl acetate (organic) layer was separated using a separating funnel. The obtained extracts were concentrated under a vacuum and dissolved at 0.1% w/v in dimethyl sulfoxide (DMSO) solution for further assays (Dos Santos *et al*., 2021).

Qualitative phytochemical analysis of organic extracts

Biochemical assays

About 3 ml of the extracts were mixed with 1 ml of 3% ferric chloride; the formation of a dark green colour indicates the presence of total phenolic compounds (Kaur and Kapoor, 2002). About 5 ml of extract was tested with a few drops of 10% ferric chloride. A blue-black or blue-green precipitate shows the presence of tannins (Efiong *et al*., 2020). To 1 ml of the filtrate, 6 drops of Mayor's reagent was added, formation of an orange precipitate indicates the presence of alkaloids. To detect saponins, 0.5 ml of the filtrate was mixed with 0.5 ml of vanillin reagent and 0.5 ml of 72% H_2SO_4 for 10 minutes. After cooling, development of brick red colour indicative of positive result. Mixing 0.5 ml of the solution with 0.1 g of metallic zinc and 8 ml of concentrated sulfuric acid, check for the creation of a red hue, which indicates the presence of flavonoids (Parekh and Chanda, 2007).

Analytical thin layer chromatography (TLC)

Presence of glycosides, arbutin alkaloids, flavonoids, saponins and coumarins separated using the solvent systems such as ethyl acetate: methanol: water (10:13.5:1), ethyl acetate: formic acid: glacial acetic acid: water (10:11:11:26), ethyl acetate: formic acid: glacial acetic acid: water (10:11:11:26), chloroform: glacial acetic acid: methanol: water (64:32:12:8), and hexane: ethyl acetate (93:7) and separated bands detected by UV or visible light (Kagan and Flythe, 2014).

Biochemical characterization of phytochemicals by non-conventional methods

The partially purified compounds from the extract were subjected to FT-IR analysis. The powdered sample was loaded in FT-IR Spectroscopy 4600 type A (JASCO, India), with a scan range from 400 to 4000 cm^{-1} with a resolution of 8 cm^{-1} at a scanning speed of 2 mm/ sec using a 3000 Hz filter (Pakkirisamy *et al*., 2017).

GC-MS analysis was carried out in a combined Agilent gas chromatography and mass spectrophotometer, fitted with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 µm × 250 µm, film thickness 0.25 µm) with Triple-Axis detector. Helium gas was used as carrier gas at a constant flow rate of 1.0 ml/min injection volume of 1 µl. GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 16.2 psi; out time, 1.8 mm; and 1 µl injector in split mode with split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 360 °C for 5 min and changed to 150V at 4 °C/min. After 23 minutes of running at 200 °C per minute, the temperature was raised to 250 °C. By comparing each component's average peak area to the overall areas, the relative percent quantity was determined. Compilation of substances identified using the National Institute of Standards and Technology standard database (NIST) (Olivia *et al*., 2021).

Quantitative assays for phytochemicals

The total phenolic content of the extract was determined by the Folin–Ciocalteu method (Kaur and Kapoor, 2002). The amount of total tannins was measured in milligrams of tannic acid equivalents/ml of sample by comparing the reaction of aluminum chloride solution with a standard tannic acid solution (Sen *et al.,* 2013). Total saponin was identified by the vanillin-sulphuric acid assay. Total flavonoid content was estimated based on the reaction with aluminum nitrate and a standard curve established with quercetin (Wu *et al*., 2001).

Antifungal assay of extracts against the M. furfur

Inoculum preparation

Fungal strains were freshly sub-cultured in sterile Sabouraud Dextrose broth (SDB) with 2% olive oil and incubated at 30 °C for 24 hours. The resultant broth was centrifuged at 10062 X g for 5 minutes, pellet washed in sterile saline, and the turbidity adjusted to 0.5 McFarland standard, equivalent to 1×10^5 CFU/ml.

Disc diffusion assay

Yeast inoculum was cultured on SDA with 2% olive oil and sterile paper discs of 6 mm diameter impregnated with 10, 20, 30, 40, and 50 µl of different organic solvent extract and fluconazole (10 µl) discs placed on the media surface. After the incubation period at 30 °C for 24 hours, the diameter of the inhibitory zone was measured, and interpreted according to the Clinical and Laboratory Standards Institute (CLSI), FDA standards.

Broth dilution assay for determination of MIC

A hundred milligram of *A. parvum* extract was dissolved in 1 ml of sterile SDB. The suspensions were mixed, and then 500 µl of the suspension was transferred aseptically into the next tube with 500 µl of SDB, which reduced the sample concentration to half. Similarly, dilutions were carried out 50, 25, 12.5, 6.25, 3.12, and 1.56 mg/ml. A tube with 500 µl of SDB and 10 mg of fluconazole was a positive control, and a tube with 500 µl of SDB was the negative control. Each tube was mixed with 50 µl (10% v/v) of overnight inoculum and incubated at 30 °C for 24hrs. After incubation, the turbidity in each test tube was measured using UV spectrophotometer (JASCO) at 600 nm (Leong *et al*., 2017). The uninoculated SDB served as a blank. Further, aseptically a loopful of inoculum from each tube was streaked on the SDA plate to assay the fungicidal activity of the drug. The plates were incubated at 30 °C for 24 hours. No turbidity in the test tube, but the presence of

growth on the petri plate indicates the fungistatic activity of the drug, no turbidity in test tubes, and no growth on the agar plate indicates the fungicidal activity of the drug. Turbidity in the tube and growth on culture media indicates the ineptness of the drug (Jacinta *et al*., 2014).

Biofilm inhibitory efficacy of onion bulb extract by crystal violet staining

The biofilm inhibitory efficacy of the onion bulb extract was evaluated by crystal violet staining using microtiter plate assay (Melo *et al.,* 2011). As stated in the broth dilution assay, the media and extract concentrations were used.

Purification of active ingredients by preparative TLC and bioautography

The methanolic extract was chromatographed on a 5 x 12 cm TLC plate. Chloroform: methanol: formic acid (8:1.5:0.5, v/v/v) was used as a solvent system. The separated bands were visualized by visible and UV (at 254 and 366 nm) light. The bands were scraped from the plate and extracted with ethyl acetate. The filtrate was analyzed for their bioactivities by bioautography assay.

Results and Discussion

Qualitative phytochemical analysis of organic extracts

From 10 g of the onion bulb sample, maximum extract of 3.05% i.e., 0.305 g obtained using methanol as solvent and other extracts yielded approximately 0.3% of extract. The collected extracts were covered with aluminum foil and stored at 4 °C until further use. Biochemical tests used for the qualitative analysis showed the presence of phenolic, tannins, flavonoids, and alkaloids in all the extract, and saponins only in methanol extract. Tannins and flavonoids were absent in acetone and water extracts (Table 1).

Table 1. Qualitative biochemical assay results for phytochemicals assay in onion bulb

Plant extract	Phenols	Tannins	Alkaloids	Saponins	Flavonoids & Flavones
APW	++	++	++	+	-
APM	+++	+++	+++	+	+++
APE	++	++	++	-	++
APC	+	+	+	-	+
APA	+	+	-	-	-

Note: P +: Low; **++**: Medium; **+++:** High concentration; **APE:** *Allium parvum* ethanol extract; **APW:** *A. parvum* aqueous extract; **APC:** *A. parvum* chloroform extract; **APA:** *A. parvum* acetone extract**; APM:** *A. parvum* methanol extract

The above results concluded that extract obtained with polar solvents such as water, methanol, and ethanol extracted phenols, tannins, saponins, alkaloids, and flavonoids, whereas non-polar (acetone and chloroform) solvents extracted phenolic, tannins, and alkaloids. So, for the extraction of hydrophilic compounds, polar solvents such as methanol, ethanol, or ethyl-acetate are recommended (Cos *et al*., 2006). Hence the non-polar solvents were useful for lipophilic substance and not suitable for separating essential components from the onion bulb. Among the polar solvents, methanol extract showed the highest degree of extraction of all compounds. In 2019, Dieu and coworkers also proposed that among the solvents tested, methanol resulted in the highest extract (33.2%) from *Severinia buxifolia*, followed by distilled water (27.0%), ethanol (12.2%), acetone (8.6%), chloroform (7.2%), and dichloromethane (4.9%), indicated that high polar solvents have the highest extraction efficiency of phytochemicals than the non-polar solvents. A solvent system extracting a large amount of drug at low volume at normal temperature is commonly recommended in pharmacological drug preparation. Water is a cheap, easily available, and nontoxic solvent

with strong polarity. It is used to extract phytochemicals with strong polarities, such as inorganic salts, saccharides, amino acids, tannins, proteins, organic acid salts, alkaloid salts, and glycosides, but has a high boiling point during separation. The advantage of methanol is that it has similar properties like water but has a lower boiling point (Khoddami *et al.,* 2013).

Compounds confirmed by analytical TLC

The presence of specific phytochemicals was identified by TLC using the different solvent systems and detectors. The analytical TLC results confirmed the biochemical test results, which showed that phenols, tannins, saponins, alkaloids (glycosides), and coumarins were present in polar solvent extracts but not in non-polar solvent extracts. Glycosides include digitoxin and acetyl digitoxin-like compounds produced by plants as defensive compounds. Cardiac glycosides are steroids that can exert specific powerful action on the cardiac muscle. These compounds are primarily valuable in the treatment of congestive heart failure. Dini *et al*. in 2008 and Esienanwan *et al*. (2019) also confirmed that onion samples consist of flavonoids, phenols, tannins, cardiac glycosides, and saponins in juice, dried, and oil forms of red onion samples (Table 2).

Table 2. Analytical TLC solvent system used for detection of phytochemicals in *A. parvum*

Extract	Detector	R_f values of the bands formed from different extracts				Detected compound
		APW	APM	APE	Positive control	
Ethyl acetate: Methanol: water (10:1.3:1)	UV light	0.98	0.98	0.98	0.98 & 0.15	Glycosides/ cardiac glycosides/ alkaloids
	Visible light	1	1	1	1 & 0.17	
	10% H_2SO_4	1	1	1	1	
Ethyl acetate: Formic acid: Glacial acetic acid (10:1:1:0.2)	UV light	0.84	0.84	0.84	0.84	Flavonoids
	Visible light	-	0.2	0.2	-	
	Aluminum chloride	-	-	-	-	
Chloroform: Glacial acetic acid: Methanol: water (6.4: 3:1:1)	UV light	0.88 & 0.17	0.88 & 0.17	0.88 & 0.17	0.88 & 0.17	Saponins
	Visible light	0.88	0.88	0.88	0.88	
	Vanillin reagent	-	-	-	-	
Toluene: Ethyl acetate (9.3:0.7)	UV light	0.98	0.98	0.98	0.98	Coumarins
	Visible light	-	-	-	-	
	Potassium cyanide & Ferric chloride	0.98 & 0.03	0.98 & 0.03	0.98 & 0.03	0.98 & 0.03	

Note: APE: *Allium parvum* ethanol extract; **APW:** *A. parvum* aqueous extract; **APM:** *A. parvum* methanol extract

Chemical characterization of compound

FT-IR analysis confirmed that the compounds in *A. parvum* purified extract are quinolones (2412 cm^{-1}), organic sulfate (1411 cm^{-1}), aromatic nitro compounds (1525 cm^{-1}), primary amines (1020 cm^{-1}), and salts of carboxylic acids (1592 cm^{-1}) (Figure 1). Quinolones possess a broad spectrum of chemotherapeutic properties and demonstrate considerable antifungal activities as well. Various quinolone derivatives have been screened for their antifungal activities, and some of them exhibit excellent potency against both drug-susceptible and drug-resistant fungi (Zhang, 2019). Organic sulfate monoesters are sulfuric acid derivatives extensively found in biological systems, such as steroids, carbohydrates, and proteins that function as surfactants (Michael, 2000). Primary amines contain an NH_2 group, and their spectra are dominated by the

stretching and bending of this moiety. Used as an anesthetic and is a very common decongestant (Sahin *et al.,* 2017).

The presence of phytochemicals responsible for antioxidant, antifungal, anti-tumor, and antagonistic surfactant activity (antimicrobial agent) was revealed by GC-MS analysis. The identified compounds are listed below in Table 3 and Figure 2. The major antimicrobial compound present in the sample was identified as diallyl sulfide, an organosulfur compound in the *A. parvum*. A yellow colour, volatile compound recognized from *A. sativum*. The highly unstable organosulfur compounds are converted to allyl sulfide and its derivatives (Rao *et al.,* 2015). These derivatives effectively inhibited the metabolic activity of the by interfering the synthesis and secretion of protease and phospholipase enzymes that controlled the growth of specifically unicellular and multicellular fungi such as *Candida albicans* and *Aspergillus versicolor* similar to controlling *M. furfur* in the present study (Song *et al*., 2021).

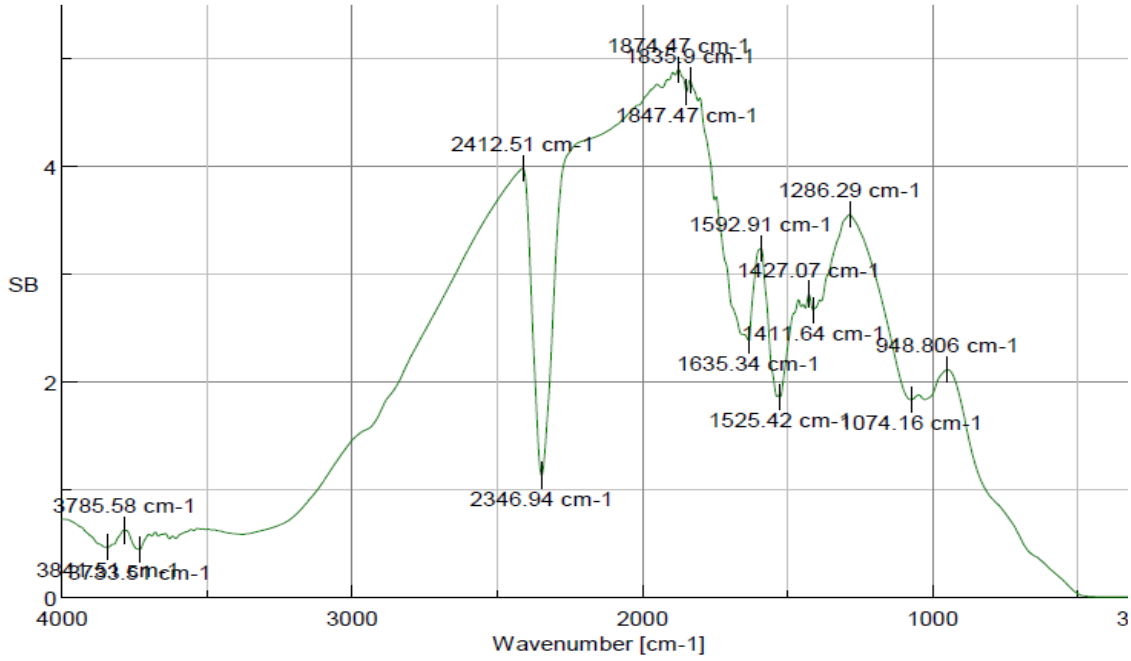

Figure 1. FT-IR analysis of methanol extract of *A. parvum*

Table 3. GC-MS spectrum of compounds from *A. parvum*

Retention time	Peak area (%)	Compound	Properties and uses	References
5.509	3.71	Butanoic acid/ Butyric acid	Essential plant oil – antihistaminic (anti-inflammatory)	Chen *et al*., 2018
7.802	2.97	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-ester	Antioxidant	Mozafari *et al.,* 2018
10.664	0.83	Diallyl sulfide	Antimicrobial mechanism of binding to thiol-containing proteins /enzymes in bacterial cells generating a disulfide stretching band and altering the α-helix and β-sheet	Feng, *et al*., 2014
11.975	29.98	2-Hexane isothiocyanate	Inhibit the proliferation of cancerous cells - Anticancer Drug	Patent No: JP-2013209314

13.552	23.12	Thiopyran terahydro or Deoxy d manic lactone	Treatment of lung cancer	Patent No: WO-2021041671-A1
13.875	5.3	D-Gluconic acid, delta-lactone / Imidazole, 2-fluoro-5-hydroxy-1-...	Antiperspirant and anticancer drug	SID 389367705,
20.551	1.78	Allyl methyl trisulfide	Flavoring agent, adjuvant and anticancer agent (eye irritant)	Yannai and Shmuel, 2004

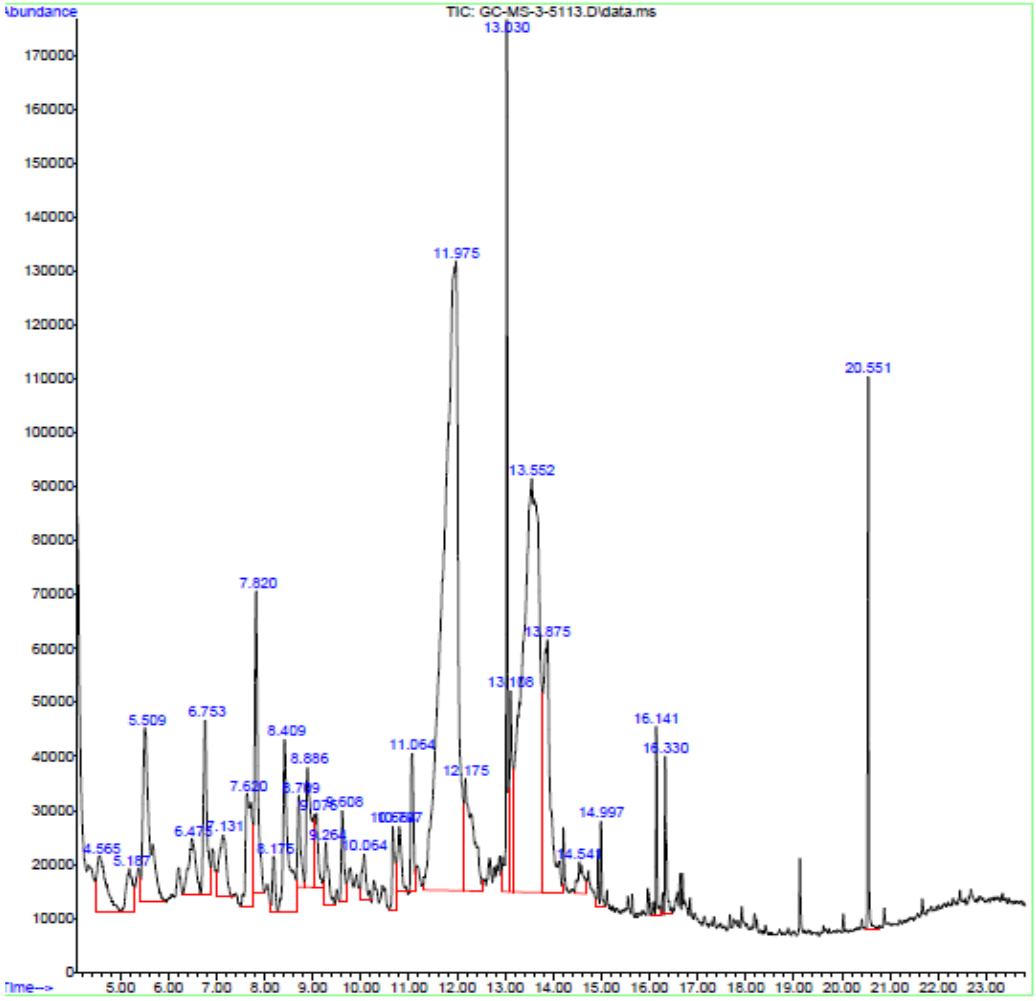

Figure 2. GC-MS spectrum of compounds from *A. parvum*

Allium cepa, red and white varieties showed antimicrobial and antioxidant activities. These are properties are curative for implications from and for food cultures for cardiovascular disease and provide longevity (Upadhyay, 2016). Onions are presumed to have antibacterial and antifungal properties, making them useful against human pathogens. It possesses good amounts of flavonoids, polyphenols, sulfur compounds, and a variety of other secondary metabolites, all of which are responsible for its therapeutic properties (Anyaegbunam *et al*., 2019).

Quantitative analysis of organic extracts

Quantitatively abundant presence of phenols and flavonoids was estimated, and at a lesser concentration of saponins, very trace levels of tannins were recorded from the obtained results (Table 4). Highest concentration of phenol (58± 0.1 mg GAE /g), flavonoids (112 ± 0.12 mg QE /g), saponins (1.45 ± 0.1mg AE/g) and tannins (4.1 ± 0.03 µg TA/g) were recorded from methanol extract of *A. parvum* which were low in other solvent extracts. Similar phytochemical results were obtained from the ethanol extract of *Allium subhirsutum* L. as flavonoids (231 ± 0.022 mg QE/g), tannins (159 ± 0.006 mg TAE/g), and phenols (4 ± 0.004 mg GAE/g) (Snoussi *et al.,* 2022).

Table 4. Results showing the quantity of phytochemicals present in onion bulbs

Extracts & estimated chemical values	Phenol (mg AE/g)	Tannins (µg TAE/g)	Saponins (mg GAE/g)	Flavonoids (mg OE/g)
Water extract of *A. parvum*	50 ± 0.1	2.1 ± 0.04	0.83 ± 0.14	58 ± 0.2
Methanol extract of *A. parvum*	58± 0.2	4.1 ± 0.02	1.45 ± 0.21	112 ± 0.1
Ethanol extract of *A. parvum*	31 ± 0.1	5 ± 0.07	0.41 ± 0.01	5 ± 0.05

Keys: AE: Atropine equivalent; TAE: Tannic acid equivalent; GAE: Gallic acid equivalent; QE: Quercetin equivalent

Antifungal activity of extracts against the M. furfur

Disc diffusion assay

By disc diffusion assay zone of clearance for methanol, ethanol, and water extract were detected, and a zone of inhibition was absent for acetone and chloroform extract. Similar results were recorded in the well-diffusion assay also. The maximum zone of inhibition recorded for methanol extract of *A. parvum* was 18 mm against *M. furfur* (Figure 3). Organic methanol and ethanol extracts of *A. parvum* showed a larger zone of inhibition against the pathogen than fluconazole. Fluconazole drug interferes in the ergosterol biosynthesis, thereby interrupting cell membrane integrity which causes lysis of fungi. The methanol extract has more than one efficient means to control the pathogen as it is proven to consist of flavonoids, phenolics, saponins, tannins, and coumarins. So, the pathogen cannot overcome the drug resistance against the extract. A possible mechanism of antifungal activity of the extract is due to the formation of free radicals on the surface of the microbes and subsequent damage to the lipids cell membrane by these radicals, which consequently lead to the leakage and breakdown the cell membrane (López *et al*., 2017).

Figure 3. Agar well-diffusion assay for organic solvent extracts

Broth dilution assay

The growth was measured in terms of turbidity at 600 nm, turbidity was absent in the first 3 test tubes having 12.5 mg/ml in *A. parvum,* and no growth was observed on petri plates till these dilutions. So, the minimal inhibitory concentration (MIC) of *A. parvum* was determined as 12.5 mg/ml of methanol extract, which is the comparatively similar to fungicidal activity of fluconazole at 10 mg/ml used in the study. A similar report by Sharma *et al.,* in 2019 revealed that MIC of diallyl sulfides as 0.63 to 25 mg/ml against the *Candida* sp., *Blastoschizomyces capitatus, Bacillus subtilis, S. aureus, E. coli* and *P. aeruginosa* pathogens (Sharma *et al.,* 2019).

Determination of minimum biofilm eradication concentration (MBEC) by crystal violet staining

In biofilm formation assay, the crystal violet staining method is adopted because it stains both the metabolically active and inactive cells in mature biofilms. So, it is considered the most appropriate, convenient, and reliable test for determining biofilm formation by the pathogen. Methanol extract of *A. parvum* inhibited the biofilm formation by *M. furfur* at 25 mg/ml concentration, so the MBEC was determined as 25 mg/ml, and standard fluconazole is 10 mg/ml. The MBEC value of 10 mg/ml of the standard fluconazole drug doubled the concentration of the drug required (Figure 4). Further purification of the drug still reduces the required dosage level.

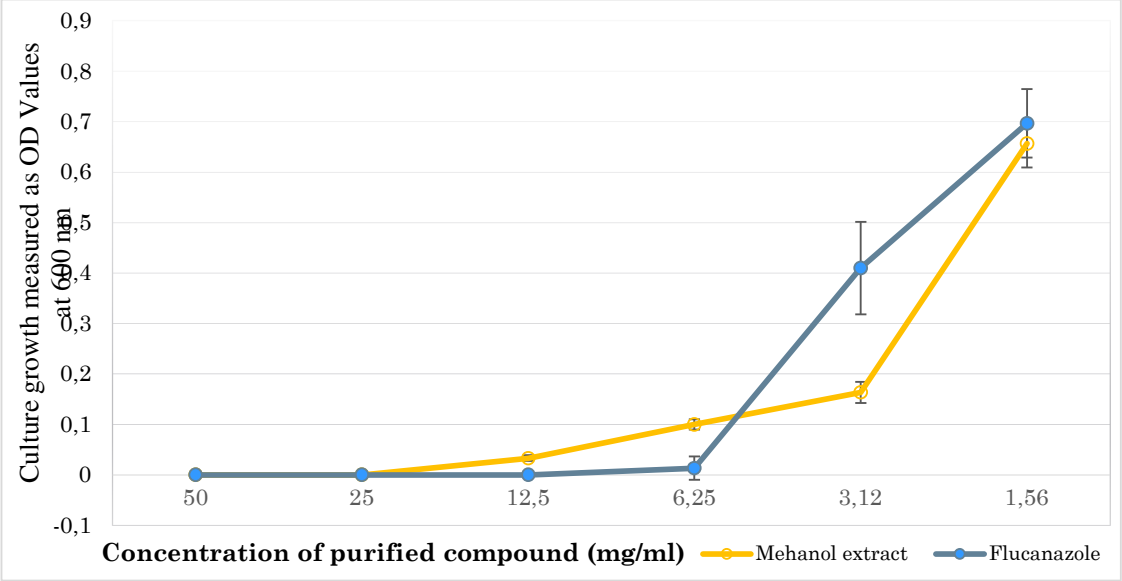

Figure 4. Standard error graph determining biofilm eradication concentration

Purification of active ingredients by preparative TLC and bioautography

The two different fractions were obtained, and the antimicrobial activity assay showed that fraction 1 from *A. parvum* showed 21 mm zone of clearance, and fraction 2 showed 14 mm zone of clearance. Upon preparative HPLC purification during drug formulation of this compound, further improvements can be made to the functional efficacy of the compound (Zhang *et al*., 2022). The fraction-1 contain antimicrobial compounds like butanoic acid/ butyric acid, 2-hexane isothiocyanate, 3-deoxy-d-mannoic lactone, allyl sulfide, cyclohexane, and butanal, 2-methyl- ester / pentanal, responsible for the fungicidal activity of the *M. furfur*.

Organic sulfur compounds such as diallyl sulfide, allyl methyl di sulfide, allyl methyl tri sulfide, thiopyran, and 2 hexane thiocyanate like compounds are proposed to alter the secondary structure of enzymes and inactivate their functions. Moreover, the butanoic acid, gluconic acid, and delta lactone-like amphipathic agents act as the bacterial cell membrane and cell wall surface-active agents and cause rupture of the cells. Strains

of *M. furfur* that form biofilms are unable to counteract the fungicidal activity of *A. parvum* due to these two potent mechanisms.

Potential thiosulphinates from *Allium* extracts react with free SH groups of intracellular enzymes and affect intracellular processes and cell communication. A complex mixture of bioactive compounds in plant extracts and their mutual interactions are common explanations for the overall observed antifungal activity in the *A. parvum*. The antimicrobial effects are also due to tannins (e.g., tannic acid) or flavonoids, inhibiting cytoplasmic membrane function, DNA synthesis, and even protein and RNA synthesis. Also, studies confirmed that 96% of ethanol extract of *A. ursinum* exhibited the strongest antioxidant, spasmolytic, and antimicrobial potential due to high polyphenols, tannins, and flavonoids that are responsible for its impact on tested enteropathogenic microorganisms (Dragana *et al*., 2017).

In garlic bulb, the presence of several organic sulfur compounds was also confirmed, and consumption of these compound are approved by the FDA as "generally recognized as safe". These organic sulfur compounds showed rapid antimicrobial activity except for *H. pylori.* As a result, the study suggests that *A. parvum* be administered in addition to overcoming drug-resistant pathogen-associated mortality. It is possible by the recent advances in combinatorial and refined chemical synthesis techniques, nanotechnology, bioinformatics, computation tools, and advanced formulation resulting in garlic and onion bulb-based organosulfur-based novel antibiotics. These organosulfur compounds warrant more development for control of biofilm-forming, drug-resistant pathogens such as *M. furfur,* thereby reducing the risk of bacterial infection-based mortality (Bhatwalkar *et al*., 2021). Especially diallyl sulfide interferes with the expression of microbial virulence factors, such as elastase, pyocyanin, and swarming motility, etc. It blocks the biofilm formation by the inactivation of quorum-sensing luxI and luxR genes in *Hafnia alvei* and *Pseudomonas aeruginosa* (Li *et al.,* 2018). *M. furfur* also be inhibited similarly and need to be analysed in the future.

Conclusions

The *A. parvum* consists of a vast number of chemical compounds like flavonoids such as quinolones, surfactants like butanoic acid and gluconic acid, phenolics, alkaloids, and different types of organic sulfur compounds that inhibit the growth of fungal pathogens like *M. furfur* effectively. The majority of phytochemicals demonstrated minimum biofilm inhibitory activity at 25 mg/ml concentrations and maximum fungicidal activity against the pathogen at a MIC of 12.5 mg/ml among the water, methanol, ethanol, hexane, and chloroform methanol extracts. Purification of the compound and the assay result in a promising drug candidate for treating *M. furfur*-associated superficial and implant-associated infections.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors thankfully acknowledge the central instrumentation facility sponsored by DST-FIST, Government of India in RVS College of Arts and Science, for providing facilities for this study.

The authors thank for sanctioning funds from Tamilnadu State Council for Science and Technology, India, to execute the project successfully.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Anyaegbunam KZ, Amaechi LO, AnyaegbunamTito C, Wisdom OO, Henrietta CO, Cosmas S, Sabinus IO (2019). Antibacterial activity of fresh red and white onion (*Allium cepa*) extract against some drug resistant bacteria. Journal of Advances in Microbiology 16(4):1-8. *https://doi.org/10.9734/jamb/2019/v16i430127*

Bhatwalkar SB, Mondal R, Krishna S, Adam JK, Govender P, Anupam R (2021). Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). Frontiers in Microbiology 12:613077. *https://doi.org/10.3389/fmicb.2021.613077*

Borda LJ, Wikramanayake TC (2015). Seborrheic dermatitis and dandruff: a comprehensive review. Journal of Clinical and Investigative Dermatology 3(2). *https://doi.org/10.13188/2373-1044.1000019*

Chen G, Ran X., Li B, Li Y, He D, Huang B, Fu, S, Liu J, Wang, W (2018). Sodium butyrate inhibits inflammation and maintains epithelium barrier integrity in a TNBS-induced inflammatory bowel disease mice model. E Bio Medicine 30:317-325. *https://doi.org/10.1016/j.ebiom.2018.03.030*

Cos P, Vlietinck AJ, Berghe DV, Maes L (2006). Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. Journal of Ethno Pharmacology 106:290-302. *https://doi.org/10.1016/j.jep.2006.04.003*

Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. Journal of Food Quality 2019. *https://doi.org/10.1155/2019/8178294*

Dini I, Tenore GC, Dini A (2008). Chemical composition, nutritional value and antioxidant properties of *Allium cepa* L. Var. *tropeana* (red onion) seeds. Food Chemistry 107:613-621. *http://dx.doi.org/10.1016/j.foodchem.2007.08.053*

Dos Santos E, Tingeira TS, da Costa VdFC, Luana Marcele Chiarellow (2021). Essential oil extraction from onion using ethanol and CO2 as an extraction fluid mixture. F1000 Research 1(1):625-635. *https://doi.org/10.12688/f1000research.52925.1*.

Pavlović DR, Veljković M, Stojanović NM, Gočmanac-Ignjatović M, Mihailov-Krstev T, Branković S, ... Radenković M (2017). Influence of different wild-garlic (*Allium ursinum*) extracts on the gastrointestinal system: spasmolytic, antimicrobial and antioxidant properties. Journal of Pharmacy and Pharmacology 69(9):1208-1218. *https://doi.org/10.1111/jphp.12746*

Drake LA, Dinehart SM, Farmer ER, Goltz RW, Graham GF, Hordinsky MK, Scher RK (1996). Guidelines of care for superficial mycotic infections of the skin: Pityriasis (tinea) versicolor. Guidelines/Outcomes Committee. American Academy of Dermatology. Journal of the American Academy of Dermatology 34(2):287-289. *https://doi.org/10.1016/s0190-9622(96)80136-8*

Efiong, EE, Akumba, LP, Chukwu, EC, Olusesan, AI, Obochi G (2020). Comparative qualitative phytochemical analysis of oil, juice and dry forms of garlic (*Allium sativum*) and different varieties of onions (*Allium cepa*) consumed in Makurdi metropolis. International Journal of Plant Physiology and Biochemistry 12(1):9-16. *http://dx.doi.org/10.5897/IJPPB2019.0285*

Feng S, Eucker TP, Holly MK, Konkel ME, Lu X, Wang S (2014). Investigating the responses of *Cronobacter sakazakii* to garlic-drived organosulfur compounds: a systematic study of pathogenic-bacterium injury by use of high-throughput whole-transcriptome sequencing and confocal micro-raman spectroscopy. Applied and Environmental Microbiology 80:959-971. *https://doi.org/10.1128/AEM.03460-13*

Iatta R, Figueredo LA, Montagna MT, Otranto D, Cafarchia C (2014). In vitro antifungal susceptibility of Malassezia furfur from bloodstream infections. Journal of Medical Microbiology 63(11):1467-1473. *https://doi.org/10.1099/jmm.0.078709-0*

Santhanam J, Abd Ghani FN, Basri DF (2014). Antifungal activity of *Jasminum sambac* against *Malassezia* sp. and non-*Malassezia* sp. isolated from human skin samples. Journal of Mycology 2014. *https://doi.org/10.1155/2014/359630*

Cappuccino JG, Sherman N (2013). Microbiology: a laboratory manual. Pearson Higher Ed.

Kagan, IA, Flythe MD (2014). Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. Journal of Visualized Experiments 85:51411. *https://doi.org/10.3791/51411*

Kaneko T, Murotani M, Ohkusu K, Sugita T, Makimura K (2012). Genetic and biological features of catheter-associated Malassezia furfur from hospitalized adults. Medical Mycology 50(1):74-80. *https://doi.org/10.3109/13693786.2011.584913*

Kaur C, HC Kapoor (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. International Journal of Food Science and Technology 37:153-161. *https://doi.org/10.1046/j.1365-2621.2002.00552.x*

Khoddami A, Wilkes MA, Roberts TH (2013). Techniques for analysis of plant phenolic compounds. Molecules (Basel, Switzerland) 18(2):2328-2375. *https://doi.org/10.3390/molecules18022328*

Leong C, Buttafuoco A, Glatz M, Bosshard PP (2017). Antifungal susceptibility testing of *Malassezia* sp., with an optimized colorimetric broth microdilution method. Journal of Clinical Microbiology 55:1883-1893. *https://doi.org/10.1128/jcm.00338-17*

Li WR, Ma YK, Shi QS, Xie XB, Sun TL, Peng H, Huang XM (2018). Diallyl disulfide from garlic oil inhibits Pseudomonas aeruginosa virulence factors by inactivating key quorum sensing genes. Applied Microbiology and Biotechnology 102(17):7555-7564. *https://doi.org/10.1007/s00253-018-9175-2*

López C, Rodríguez-Páez JE (2017). Synthesis and characterization of ZnO nanoparticles effect of solvent and antifungal capacity of NPs obtained in ethylene glycol. Applied Physics A 123:748. https://doi.org/10.1007/ s0033ƛ-017-133ƛ-x

Melo, AS, Bizerra FC, Freymüller E, Arthington-Skaggs BA, Colombo AL (2011). Biofilm production and evaluation of antifungal susceptibility amongst clinical Candida spp. isolates, including strains of the *Candida parapsilosis* complex. Medical Mycology 49(3):253-262. *https://doi.org/10.3109/13693786.2010.530032*

Meyers E, Erickson RC (1967). Bioautography of antibiotics on thin layer chromatograms. Journal of Chromatography 26:531-532. *https://doi.org/10.1016/s0021-9673(00)86603-0*

Michael A Kertesz (2000). Riding the sulfur cycle – metabolism of sulfonates and sulfate esters in Gram-negative bacteria. FEMS Microbiology Reviews 24(2):135-175. *https://doi.org/10.1016/S0168-6445(99)00033-9*

Mnayer D, Fabiano-Tixier AS, Petitcolas, E, Hamieh T, Nehme N, Ferrant C, Fernandez X, Chemat F (2014). Chemical composition, antibacterial and antioxidant activities of six essentials oils from the Alliaceae family. Molecules (Basel, Switzerland) 19(12):20034-20053. *https://doi.org/10.3390/molecules191220034*

Mozafari AA, Vafaee Y, Shahyad M (2018). Phytochemical composition and in vitro antioxidant potential of *Cynodon dactylon* leaf and rhizome extracts as affected by drying methods and temperatures. Journal of Food Science and Technology 55:2220-2229. *https://doi.org/10.1007/s13197-018-3139-5*

National Center for Biotechnology Information (2022). PubChem Patent Summary for JP-2013209314-A. Retrieved from: *https://pubchem.ncbi.nlm.nih.gov/patent/JP-2013209314-A*.

National Center for Biotechnology Information (2021). PubChem Patent Summary for WO-2021041671-A1, Kras g12d inhibitors. Retrieved from: *https://pubchem.ncbi.nlm.nih.gov/patent/WO-2021041671-A1*.

National Center for Biotechnology Information. PubChem Substance Record for SID 389367705, SID 389367705, Source: PATENTSCOPE (WIPO). *https://pubchem.ncbi.nlm.nih.gov/substance/389367705*.

Olivia NU, Goodness UC, Obinna OM (2021). Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves. Future Journal of Pharmaceutical Sciences 7:1-5. *https://doi.org/10.1186/s43094-021-00208-4*

Pakkirisamy M, Kalakandan SK and Ravichandran K (2017). Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacognosy Journal 9(6):952-956. *http://dx.doi.org/10.5530/pj.2017.6.149*

Parekh, J, Chanda S (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research 10:175-181. *http://dx.doi.org/10.4314/ajbr.v10i2.50624*

Rao PS, Midde NM, Miller DD, Chauhan S, Kumar A, Kumar S (2015). Diallyl Sulfide: potential use in novel therapeutic interventions in alcohol, drugs, and disease mediated cellular toxicity by targeting cytochrome P450 2E1. Current Drug Metabolism 16(6):486-503. *https://doi.org/10.2174/1389200216666150812123554*

Roques C, Brousse S, Panizzutti C (2006). *In vitro* antifungal efficacy of ciclopirox olamine alone and associated with zinc pyrithione compared to ketoconazole against *Malassezia globosa* and *Malassezia restricta* reference strains. Mycopathologia 162(6):395-400. *https://doi.org/10.1007/s11046-006-0075-0*

Şahin Mİ, Kökoğlu K, Güleç Ş, Ketenci İ, Ünlü Y (2017). Premedication methods in nasal endoscopy: a prospective, randomized, double-blind study. Clinical and Experimental Otorhinolaryngology 10(2):158-163. *https://doi.org/10.21053/ceo.2016.00563*

Sen S, De B, Devanna N, Chakraborty R (2013). Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of *Meyna spinosa* Roxb., an Indian medicinal plant. Chinese Journal of Natural Medicines 11(2):149-157. *https://doi.org/10.1016/s1875-5364(13)60042-4*

Sharma P, Sidhu A (2019). Review article on: Synthesis and biological activities of allyl sulfides. PharmaTutor 7(8):79-97.

Snoussi M, Noumi E, Hajlaoui H, Bouslama L, Hamdi A, Saeed M, ... Kadri A (2022). Phytochemical profiling of *Allium subhirsutum* L. aqueous extract with antioxidant, antimicrobial, antibiofilm, and anti-quorum sensing properties: *In vitro* and *in silico* studies. Plants 11(4):495. *https://doi.org/10.3390/plants11040495*

Song X, Yue Z, Nie L, Zhao P, Zhu K, Wang Q (2021). Biological functions of diallyl disulfide, a garlic-derived natural organic sulfur compound. Evidence Based Complementary and Alternative Medicine 5103626:29. *https://doi.org/10.1155/2021/5103626*

Teshika JD, Zakariyyah AM, Zaynab T, Zengin G, Rengasamy KR, Pandian SK, Fawzi MM (2019). Traditional and modern uses of onion bulb (*Allium cepa* L.): a systematic review. Critical Reviews in Food Science and Nutrition 59:S39-S70. *https://doi.org/10.1080/10408398.2018.1499074*

Tragiannidis A, Bisping G, Koehler G, Groll AH (2010). Minireview: Malassezia infections in immunocompromised patients. Mycoses 53(3):187-195. *https://doi.org/10.1111/j.1439-0507.2009.01814.x*

Upadhyay, Ravi (2016). Nutraceutical, pharmaceutical and therapeutic uses of *Allium cepa*: A review. International Journal of Green Pharmacy 10:46-64. *https://doi.org/10.22377/ijgp.v10i1.612*

Velavan S (2015). Phytochemicla techniques – a Review. World Journal of Science and Research 1(2):80-91.

Vest BE, Krauland K. *Malassezia furfur* (2022). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. PMID: 31971731. *https://www.ncbi.nlm.nih.gov/books/NBK553091/*

Wu J, Lin L, Chau FT (2001). Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. Ultrasonics Sonochemistry 8:347-352. *https://doi.org/10.1016/s1350-4177(01)00066-9*

Yadav R, Agarwala M (2011). Phytochemical analysis of some medicinal plants. Journal of Phytology 3:104.

Yannai S (2004) Dictionary of food compounds with CD-ROM: Additives, flavors, and ingredients. Boca Raton: Chapman & Hall/CRC.

Zhang B (2019). Quinolone derivatives and their antifungal activities: An overview. Archives der Pharmazie 352(5):e1800382. *https://doi.org/10.1002/ardp.201800382*

Zhang Y, Zhao Z, Li W, Tang Y, Meng H, Wang S (2022). Purification of two Taxanes from *Taxus cuspidata* by preparative high-performance liquid chromatography. Separations 9(12):446. *https://doi.org/10.3390/separations9120446*

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.