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Contents

Biomimetic Approach to Phyto-Mediated Synthesis of Zinc Oxide Nanoparticles from Waltheria Indica Root Extract and Exploring their Potential In-Vitro Antibactertial Activity

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ABSTRACT

This study investigates biogenic ZnO NPs synthesised from Waltheria indica root extract and their antibacterial properties. UV-visible, FTIR, XRD, and SEM-EDAX confirmed ZnO NP formation. UV-Vis spectrophotometers show ZnO NP formation with a 374 nm SPR peak. XRD confirmed the NPs' structural crystallinity. SEM micrographs show hexagonal ZnO Nps. W. indica root extract-mediated ZnO NPs from biological synthesis inhibited many microbial strains. The study found that ZnO NPs have antibacterial properties against all tested microorganisms, with a 13-mm zone of inhibition for S. flexneri at 100 µg/mL concentration. This suggests that biologically synthesised ZnO nanoparticles may be utilised in therapeutics to improve effectiveness, owing to their significant antibacterial properties. Moreover, further investigation may reveal new opportunities for developing innovative and secure treatments for bacterial infections through the use of biofabricated ZnO nanoparticles.

KEYWORDS *Biofabrication, Waltheria indica roots, FTIR, XRD, SEM-EDAX, In-vitro antibacterial activity.*

INTRODUCTION

Nanotechnology is one of quickly fascinating interdisciplinary research, chemistry, aerospace etc. The objective encompasses the synthesis and application of nanoparticles, characterized by at least one dimension ranging from 1 to 100 nm. This study examines these particles in terms of design, manufacturing, characterization, and the application of small functional systems derived from these materials. These nanoparticles exhibit 0D, 1D, 2D, and 3D structures, contingent upon their size [1]. The optical property is a principal and fundamental characteristic of nanoparticles. The importance of these nanoparticles became evident when researchers discovered that size influences the physicochemical properties of the material. The active surface phenomenon and increased surface area of metal nanoparticles have demonstrated effectiveness in biomedical research. Nonetheless, the fundamental potential characteristics of nanoparticles (metals) are inherently enduring, and their surface properties can be altered based on their application in Due to the stable extensive properties are unique properties of

the (metal) nanoparticle these can be used in various fields. Nanoparticles can be categorized into various types based on their morphology, size, and shape; several examples are provided below. Organicnanoparticles. Inorganic nanoparticles include metal nanoparticles, ceramic nanoparticles, metal oxide nanoparticles, and biological nanoparticles, also referred to as bio nanoparticles [3]. Metallic nanoparticles synthesized through biological methods find applications in biomedical fields, including protection against harmful microorganisms, bio-mining, therapies for cancer, and healthcare diagnosis, their unique properties, such as anti-metastasis, biocompatibility, stability, and manipulability, enhance their utility. Additionally, metallic nanoparticles exhibit catalytic activity, making them significant in various industrial applications in contemporary settings [4].

The synthesis of nanoparticles can involve either natural or synthetic origins, resulting in materials that exhibit unique properties at the nanoscale. Two fundamental approaches exist: the top-down approach and the bottom-up approach. In the top-down approach, nanoparticles are produced through size reduction, which is accomplished using various physical and chemical methods [5]. In bottom up approach nanoparticles are synthesized by gathering and combing liquid atoms or molecules, where the main reaction is reduction or oxidation. But these methods are rarely preferred due to their relative pros and cons, so phytonanotechnology the synthesis of nanoparticles using fresh plants or plant extract or microorganisms, also known as green or biological synthesis is the best. Researchers prefer biological nanoparticle synthesis because it is eco-friendly, simple, economical, and reproducible for faster metal nanoparticle production [6, 7].

The green synthesis method represents a bottom-up approach akin to chemical reduction, wherein costly reducing agents are substituted with extracts from natural sources, such as tree leaves or fruit crops, for the synthesis of metals or metal oxides. Additionally, microorganisms may also be employed in this process. Among biological entities, plants or plant extracts appear to be the most effective agents due to their accessibility, suitability for large-scale nanoparticle production, and the eco-friendly nature of their waste products [4, 8]. Plants or their extract are rapidly used as a substitute of chemical reducing agent in the production of nanoparticle because of their heavy metal accumulation, detoxification and reduction properties and the secondary metabolites in the plants also acts as reducing agent. Zinc oxide, gold, silver nanoparticles are already produced and they are used in various fields [9].

The biological approach of reducing metal precursors to create corresponding nanoparticles is environmentally friendly, cost-effective, and devoid of chemical contaminants. This method is particularly suitable for medical and natural applications, where the purity of the NPs is of utmost significance. An alternative synthesis technique should be developed, that avoids the use of hazardous and toxic substances. Therefore, Biosynthesis of Nanoparticles is more advantageous than physicochemical approaches [5]. While numerous nanoparticles have been utilised for many purposes, Zinc oxide nanoparticles (ZnO NPs) have garnered the highest level of interest due to their potential applications in cancer treatment and diagnosis [2, 8]. These nanomaterials are highly important and intriguing nanoparticles used in bio-medical applications. Due to their distinctive physic-chemical characteristics, they are considered highly attractive nanomaterials in the field. The ZnO NPs produced through biosynthesis possess distinct biological characteristics, including pronounced scattering, minute particle size, and a large surface area. ZnO NPs have been found to possess morphological structures that can effectively combat infections in wounds and burns. This is likely due to the Ag component, which provides ZnO NPs with antibacterial, antifungal, anti-platelet, and antiviral properties [10, 11].

Similar findings were made regarding the biosynthesis and characterization of zinc oxide nanoparticles using a plant extract from Cinnamomum verum that facilitated green synthesis. Samples that were prepared were validated for their nanoscale dimensions through the application of sophisticated

characterization methods, including powder XRD and various microscopic techniques, specifically SEM and TEM. The SEM images illustrate the distinct agglomeration of particles, a finding that was corroborated by TEM studies. The green synthesized ZnO nanoparticles demonstrated inhibitory effects on the growth of E. coli and S. aureus, with minimum inhibitory concentrations (MIC) recorded at 125 µg/mL and 62.5 µg/mL, respectively. The findings suggest that the synthesized ZnO nanoparticles may serve as a viable antimicrobial agent against pathogenic microorganisms [12]. Previous studies found that they successfully synthesized the, synthesis of ZnO nanoparticles utilizing the extract from the seeds and barks of Azadirachta indica for antibacterial applications is supported by XRD patterns and FTIR spectra, which confirm the hexagonal wurtzite crystalline structure and the photochemical coating of the nanoparticles, respectively. The antimicrobial activity of nanoparticles is examined against four bacterial strains and it was observed that ZnO nanoparticles exhibit a greater inhibitory effect on the growth of gram-positive microbes compared to gram-negative microbes, as indicated by the minimum inhibitory concentration (MIC) values [13].

Waltheria indica is highly valued medicinal plant used to treat the cancer. W. indica is a species of flowering plant in the mallow family Malvaceae that has pan tropical distribution. It is believed to have originated in the Neotropics. W. indica also known as Velvet leaf, Marsh yellow, Monkey bush, and many other names. It is found throughout the tropics and warmer sub tropics. W. indica is a plant growing in many regions of the world. It has been used in traditional medicine for the treatment of several diseases in Hawaii and South Africa [14, 15].

To best of our knowledge, till now there is no research has been done on the bio-friendly production of ZnO NPs using Waltheria indica and its parts. As a result, the objective of this research work was to synthesise ZnO NPs by utilizing W. indica roots extract, and to assess their potential for in vitro antibacterial activity.

2.Material and methods

Collection of chemicals, plant sample and pathogens

Zinc nitrate hexahydrate Zn (NO3)2 6H2O was purchased from Hi-media laboratory, Mumbai Pvt Ltd, India. W. indica (Figure. 1) were collected from the surroundings of Chandravalli forest, Chitradurga and the plant specimen was authenticated by the institution taxonomist (Voucher No: KU/BOT/2021-22/MALVSNKNS-08). The microbial pathogens such as, E. coli, S. flexneri, B. subtilis, and S. aureus were procured from IMTECH, Chandigarh, India.

Figure 1: Morphology of *Waltheria indica* A) Whole Plant, B) Stem, C) Leaves, D) Unopened Flowers, E) Flowers, and F) Fruits.

Preparation of W. indica roots extract

The collected roots were removed and properly cleaned three times with tap water and with sterile distilled water, and subsequently permitted to air dry in a shaded environment for a duration of ten

days. The fine mixture was prepared using an electric blender and subsequently stored in zip-lock polythene bags. Roots that had been ground into a fine powder were roughly weighed (25 g), mixed with 500 mL of distilled water, and then boiled at 80 °C for about 5 hours. The obtained mixture was filtered using Whatman No. 1 filter paper, and the prepared filtrate was kept at 4 °C for further experimental use [12].

Synthesis of ZnO nanoparticles

2 grams of concentrated W. indica root extract were dissolved in 100 mL of distilled water. Precisely 2.9 grams of Zn(NO3)2·6H2O were combined with 5 milliliters of diluted plant extract. The mixture was maintained in a pre-heated muffle furnace at 400 °C and underwent combustion. The reaction was completed
within a duration of 5 minutes. The synthesis of nanoparticles was conducted using varying concentrations of the plant extract, specifically 10, 15, 20, and 25 mL. The resultant product was maintained in an airtight container until subsequent utilization (Ogunyemi et al., 2019) [16].

Characterization of synthesized ZnO nanoparticles

A spectrophotometer (Double beam: METASH UV9600A) was used to scan the particles for UV-Visible spectrum in the range of 300 and 700 nm to measure ZnO NPs (Nayaka et al., 2020) [17]. The synthesised NPs were analysed using FTIR to recognize the functional groups that cap and stabilise the nanoparticles. Water was removed from W. indica root extract mediated ZnO NPs by thermostatic desiccation at 40 °C for 12 h. They were then finely mixed with 5% KBr and compressed into thin discs. The Thermo Fisher Scientific NICOLET 6700 instrument measured the transmittance spectrum of ZnO NPs. For powder crystal size determination, the power level was 40 Kv with 30 mA, and the diffraction angle was 20º to 80º for twotheta (2θ). Measurements of X-ray intensities and scattering angles determined the crystallographic structure. From the XRD pattern using Rigaku Miniflex 600, Smart-Lab SE XRD instrument. ZnONPs were characterised using SEM and EDAX (VEGA3, TESCAN) for morphological features and to check the topology of synthesised ZnO Nps.

Antibacterial nanoparticles activity ofsynthesized ZnO

The agar well diffusion method described by Nagaraja et al., (2023) [18] was used to test the synthesised ZnO NPs antibacterial activity against selected two Gram negative and two Gram positive bacterial strains, such as, S. flexneri (MTCC 1457) and E. coli (MTCC 40), B. subtilis (MTCC 6633) and S. aureus (MTCC 6908) were chosen for activity testing, respectively. A sterile cotton swab spread each culture evenly on nutrient agar plates. A gel-hole puncher created 6 mm wells in 4 mm agar plates. The wells were loaded with ZnO NPs at 25 to 100 µg/µL concentration. For 24 h, the plates were incubated at 37 ℃. Streptomycin was used as positive control. With a transparent ruler, the inhibition zone around each well was measured in millimetres after incubation.

Statistical analysis

The experiments were administered in triplicate, and the data is furnished as the mean value along with the standard deviation. The analyses were performed using Excel (2010) and OriginPro 2022b software.

3. Results and Discussion

Biosynthesis of ZnO nanoparticles and their characterizations

The synthesis of zinc oxide nanoparticles utilising aqueous W. indica root extract was validated through visual observation. The combination of colourless zinc nitrate with brown root extract resulted in the formation of a yellowish-white suspension, thereby indicating the successful synthesis of zinc oxide nanoparticles. The notable alteration in colour provided a definitive signal that the synthesis of ZnO nanoparticles had taken place (Figure 2A-C). UV-Visible spectroscopy was conducted on the W. indica root extract mediated ZnO NPs and the reacted solution to verify the production of NPs. The presence of a distinct SPR band at 374 nm confirmed the synthesis of W. indica root extract mediated ZnO NPs in the reaction mixture and it is depicted in Figure 3. The synthesis methods for green nanoparticles were highly reliable, efficient, easily accessible, and environmentally sustainable. The synthesis of Nps using this technique has garnered prominent attention from researchers across various disciplines, including medicine [19, 20].

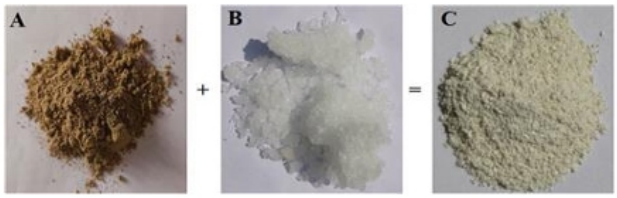

Figure 2: Visual observation of ZnO NPs. A) Root extract B) Zn $(NO_3)_2 \cdot 6H_2O$ C) Formation of ZnO NPs.

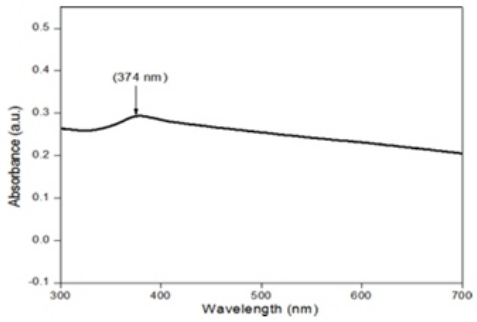

Figure 3: UV-Visible absorption spectrum of synthesised ZnO NPs from *W. indica* root extract.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR analysis identified the likely bio-molecules responsible for capping the ZnO NPs in W. indica root, which effectively stabilised the ZnO NPs (Figure 4), where 8 vibrational peaks in the 4000 to 400 cm-1. The analysis of the ZnO NPs using spectral analysis reveals noticeable changes. The peak 3281 cm-1 can be corresponded to the strectching of alcohol (O-H) bonds. Likewise, the existence of alkane stretching (C-H) is confirmed by the band detected at 2926 cm-1 in the spectrum. The presence of a (C=C) strectching cyclic alkene is indicated by the noticed band ranging from cm-1.The band at 1629 cm-1 to 1378 cm-1 suggests the bending of the (O-H) group in phenol and the bending of the (C=H)

group in alkane. The small shift in the band position of 1022 cm-1 suggests involvement primary alcohol with stretching. The vibrational bands notified at band's position at 427 cm-1 noticed to be caused by the strectching of the (C=C) bending in trisubstituted alkenes. The noticed changes in the bands of FTIR spectra correlate that the functional groups of bio constituents play a crucial role in the synthesis of Nps from the extract of plant parts. Following bio-reduction, the absorption bands indicated the presence of ZnO NPs, which were coated with bio-moieties. In a study conducted by Razanamahandry et al., (2020) and ElBelely et al., (2021), the researchers observed how the functional groups of plants interacted with biological components, resulting in the reduction of metal salts to NPs [21, 22].

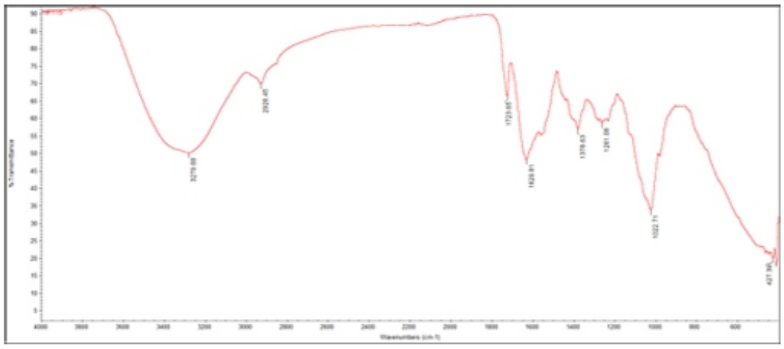

Figure 4: FTIR analysis spectrum of synthesized ZnO NPs from *W. indica* root extract.

X-ray Diffraction Analysis

X-ray diffraction (XRD) measurement was ustilised to the dimensions and morphology of ZnO Nps crystals. The synthesised ZnO NPs using the roots extract of θ were analysed using XRD patterns, which showed distinct peaks (Figure 5). An XRD investigation affirmed the crystalline property of ZnO NPs, which exhibited prominent bands at 31.74°, 34.38°, 36.30°, 47.51°, 56.61°, 62.81°, 66.36°, 68.98°, 69.10°, can be indexed to the (100), (102), (101), (102), (110), (103), (112) planes of ZnO. In a similar manner, the synthesis of ZnO nanoparticles utilizing orange peels resulted in comparable XRD patterns, exhibiting analogous Bragg reflection indices at specific 2θ angles. These findings correspond to reference code number 01-075-9742, which revealed a hexagonal structure, with no additional phases detected [23].

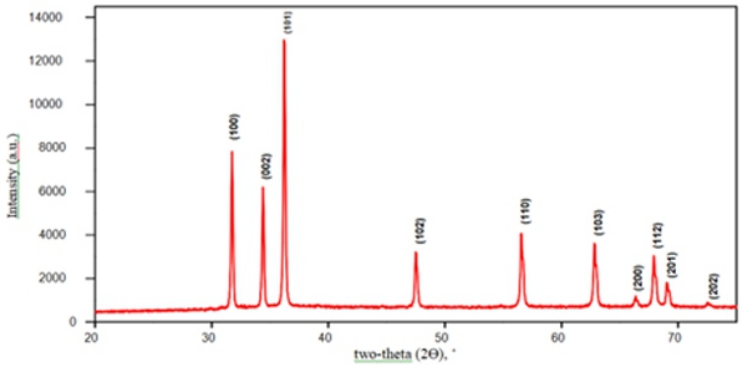

Figure 5: XRD analysis spectrum of biosynthesized ZnO NPs from *W. indica* root extract.

Antibacterial activity ofsynthesized ZnO nanoparticles

Synthesised ZnO NP's antibacterial activity was
assessed using the agar well diffusion assay. Culture plates showed circular inhibition zones. W. indica root extract mediated ZnO NPs treated Gram-positive and Gram-negative. According to the results, synthesised ZnO NPs showed the maximum growth inhibition for when treated with 100 µL of synthesised ZnO NPs, S. flexneri and E. coli showed the highest growth inhibition with zones measuring 13 mm, and 11 mm, respectively. S. auerus and B. subtilis, showed the lowest growth inhibition with zones measuring 10 mm and 9 mm. Figure 8(A-D) displayed zone of inhibition against tested pathogens in various concentrations. Overall results revealed that, metal complexes are an effective antibacterial agent for bacterial infection control. The simultaneous release of ions to bioactive compounds increased the suppression of bacterial growth at low levels [26, 27].

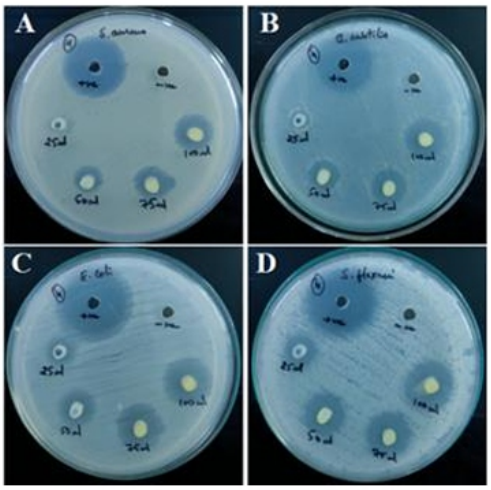

Figure 8: Antibacterial activity of different concentration of synthesised ZnO NPs from *W. indica* root extract: A) *S. aureus,* B) *B. subtilis*, C) *E. coli.* and D) *S. flexneri.*

4. Conclusion

A green synthesis method using W. indica roots extract as the reducing agent produced ZnO nanoparticles in this study. The annealing temperature and synthesis pH greatly affected ZnO nanoparticle microstructure, morphology, and bactericidal activity against S. aureus, B. subtilis, E. coli, and S. flexneri. ZnO nanoparticles show significant antibacterial activity against all tested microorganisms, with S. flexneri showing the highest inhibition zone at 100 µg/mL (13 mm). The study found that zinc oxide nanoparticles (ZnO NPs) kill bacteria by damaging DNA and cell walls through cell membrane interactions with reactive oxygen species (ROS). This study proposes a green synthesis of ZnO nanoparticles using root extract, which may reduce chemical use and nanoparticle production costs.

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6 Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interests among them.

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Exploring Caffeine Withdrawal Insomnia Using Insomnia Severity Index (Isi) Method: An Observational Study in Indian Rural Population

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ABSTRACT

Purpose

The aim of this study was to assess the physical and mental changes in individuals from a rural Indian population before and after caffeine withdrawal. This study focuses on the behavioral effects, especially insomnia, after caffeine deprivation among participants consuming high levels of caffeine daily.

Methods

An observational cross-sectional study was conducted over a two-month period among 68 volunteers at Sanjivani Rural Education Society, Maharashtra, India. Participants were selected based on their daily caffeine intake, with inclusion criteria focusing on individuals consuming more than 400 mg of caffeine per day. Data on demographics and caffeine consumption were collected using a structured questionnaire. The Insomnia Severity Index (ISI) was used to assess changes in sleep patterns before and after 72 hours of caffeine withdrawal. A multiple correlation method was applied to analyze the relationship between caffeine intake and insomnia severity.

Result

Out of 68 participants, 84% (n=59) reported sleep disturbances during the caffeine withdrawal period, which was classified as substance-induced insomnia according to DSM-IV criteria. A multiple correlation analysis indicated a mild positive association (R=0.22) between high caffeine consumption and the severity of insomnia during withdrawal. These findings suggest that excessive caffeine intake significantly affects sleep quality, particularly during withdrawal periods.

Conclusion

The study identified a notable impact of high caffeine intake on sleep disturbances in a rural Indian population. The results emphasize the importance of moderating daily caffeine consumption to prevent insomnia and improve sleep hygiene. Individualized recommendations for daily caffeine intake are crucial for mitigating potential adverse effects on sleep and overall health.

KEYWORDS *Caffeine consumption, Insomnia, Caffeine withdrawal, Rural population, DSMIV*

Introduction

Coffee is one of the most popular beverages in the world (Cano-Marquina, 2013). Tea, a beverage made from the leaves of the tea plant, originated in ancient China and has gained worldwide popularity in recent years (Y.-J. Guo, 2017). Fifteen major compounds have been identified in coffee, divided into three groups: purine alkaloids (caffeine, theobromine, trigonelline, and niacin), polyphenols (chlorogenic acid, caffeic acid, quinic acid, ferulic acid, hydroxyhydroquinone, quercetin, phenylindarins), and secoisolpenol (secoisolene). Many bioactive substances, including polyphenols, pigments, polysaccharides, alkaloids, free amino acids, and saponins, have been found in tea leaves and their brewing process (Bi, 2016; L. Guo, 2016; Pan, 2017; Wang, 2017; Tang, 2019; Zhao, 2019). These active ingredients do not contain chlorogenic acid, quinic acid, and phenylindanes due to their intestinal absorption or higher fractional bioavailability than pharmacokinetic data. Due to thermal instability, chlorogenic acids break down almost completely into volatile phenolic compounds under intense conditions (Y.-J. Guo, 2017). According to the production process, tea can be divided into six groups: white tea, green tea, yellow tea, oolong tea, black tea, and dark tea. White and green tea are not fermented, while oolong, black, and dark tea are heavily fermented (Islam, 2018; Kujawska, 2016; Lv, 2017; Sanlier, 2018; Hilal & Engelhardt, 2007; Zheng, 2015). Several studies have shown that tea and its bioactive components have many health benefits, such as anti-oxidation, anti- inflammatory, anti-cancer, anti-cardiovascular, anti diabetic, and anti-hyperlipidemia (Lorenzo; Gan, 2018; Li, 2016; Ramadan, 2017; Santamarina, 2015; Suzuki, 2016).

Coffee consumption is associated with an increased risk of many diseases, including diabetes, heart disease, neurodegenerative diseases, and cancer (CanoMarquina, 2013). Drinking coffee has also been associated with a reduced risk of Parkinson's disease. Drinking more coffee has been shown to reduce the risk of Parkinson's disease in a dose-dependent manner (Wei, 2012). Caffeine is rapidly absorbed after consumption, 99% within 45 minutes. Peak plasma concentrations occur 15 to 120 minutes after oral ingestion and may be affected by the route of administration or other foods. When caffeine is absorbed, it is quickly converted to water in the body. However, caffeine is still lipophilic enough to easily cross all biological barriers, including the blood-brain barrier. The average half-life of caffeine in the plasma of healthy people is about 5 hours, but the half-life can be between 1.5 and 9.5 hours. This large variation in exposure half-life may be due to individual differences in excretion or due to individuals smoking (shortening half-life) or using oral contraceptives (prolonging halflife) (Ferré, 2008; Fisone, 2004).

The mechanism of action of caffeine involves intracellular calcium mobilization. Some effects of caffeine on skeletal muscle appear to be related to ionized calcium (Ca++). Studies have shown that high doses of caffeine (1-10 mM) inhibit calcium uptake and its storage in the sarcoplasmic reticulum of striated muscle and increase Ca++ translocation through the plasma membrane. Caffeine can increase the Ca++ sensitivity of myofilaments by binding to ryanodine receptors on calcium channels in muscles

and Although caffeine has been shown to release calcium from intracellular stores in bone and muscle, the initial concentration required to observe this effect (250M) is greater than that required for cardiovascular disease in vivo (50M). Therefore, the subcellular effect of caffeine may not be physiologically significant (Kerrigan & Lindsey, 2005). As with most drugs, caffeine has a long list of side effects, ranging from mild to severe or even fatal, usually related to dosage and individual sensitivity. Death from caffeine toxicity is usually associated with cardiac arrhythmias, hypotension, myocardial infarction, electrolyte disturbances, and aspiration.

In 1994, a study of hundreds of physicians in Minnesota and Vermont found that 94% recommended reducing or limiting caffeine intake for patients suffering from heartburn. Jeffrey Goldberg described the findings as "difficult" and noted that a workshop was held to review whether the evidence supports the recommendations. It's not uncommon for 94% of doctors to recommend something after a heart attack, such as beta-blockers or atrial fibrillation drugs, even if the results have been demonstrated in clinical trials. While there are many ways to measure the effects of caffeine on the heart, such as QRS duration and the time it takes for the ventricles to depolarize, there are also studies that focus on three types of arrhythmia, such as atrial fibrillation, premature ventricular complexes, and arrhythmias that can cause sudden cardiac death (Hughes, 1988).

More than 93% of the adult U.S. population consumes approximately 200 mg of caffeine per day (SajadiErnazarova, 2023). In China, male and female caffeine intake was found to be 123 mg and 116 mg per day, respectively (Meredith, 2013). In Japan, 43% of the population consumes caffeine-related drinks. About 82% of the Russian population has a caffeine consumption habit (Martinchik, 2005). The Canadian population consumes an average of 400 mg/day of caffeine (Verster & Koenig, 2018). In the UAE, the per capita intake of caffeine is identified as 3.5 kg. In India, caffeine intake varies by state, and while exact data is unknown, the population in Maharashtra consumes approximately 200 mg of coffee and 150 mg of tea per day (Silverman, 1992). Yet currently, there is existing research on caffeine withdrawal studies conducted in the rural Indian population. This study specifically focuses on examining the physical and mental changes in individuals before and after caffeine withdrawal for a given time period, based on strong evidence.

Methodology

An observational cross-sectional study was conducted at Sanjivani Rural Education Society, Maharashtra, India, over a period of two months. The study included 68 participants, who were selected based on their daily caffeine consumption. The primary objective was to examine behavioral and symptomatic changes in individuals after caffeine withdrawal. The participants were chosen using a simple random sampling method, with eligibility based on their caffeine intake. The inclusion criterion for the study was a daily intake of more than six cups of caffeinated beverages (exceeding 400 mg/day). This threshold was selected based on prior research, which has shown that consuming over six cups per day can lead to severe health issues such as dementia, depression, confusion, and bradycardia. Those consuming less than six cups of coffee or tea were excluded from the study, as such intake levels are generally considered safe. A data collection form was used to gather essential demographic information, including age, gender, weight, medical history, and daily coffee consumption. The participants were between the ages of 18 and 60, with data collected on their medical backgrounds to better understand the impact of high caffeine intake. Once the participants were enrolled and informed about the study, they were given time to decide whether they wanted to participate. Those who agreed provided signed informed consent. Behavioral changes were analyzed through a series of questions based on the Insomnia Severity Index (ISI) method (Williamson, 2020; Bastien, 2001).The primary focus of the

the study was to assess the changes in participants' behavior and symptoms before and after caffeine withdrawal. Participants underwent a 72-hour period without caffeine, during which their symptoms were monitored closely. To analyze the outcomes, the study employed multiple correlation analysis, using Equation No. 1, which was designed to assess the relationships between various symptomatic changes and the duration of caffeine deprivation. Scatter plots were also generated to visually validate the associations between different variables. The study's findings were evaluated by analyzing the correlation between the variables (e.g., symptomatic changes) and the length of caffeine withdrawal. The use of statistical tools, such as multiple correlation and scatter plot analysis, enabled the researchers to assess whether there were significant behavioral and symptomatic changes in the participants after caffeine deprivation. This study provides insights into the effects of high caffeine consumption and withdrawal, focusing on the associated behavioral changes, and contributes valuable data regarding the health implications of excessive caffeine intake.

Where...R = Correlation Coefficient (R= 0 - 0.25;
Mild Positive
association, R= 0.25 – 0.5;
Moderate Positive association, R= 0.5 - 0.9;
Strong positive association.)
Y = Independent Variable

$$R = \frac{\sqrt{r}2yx1 + r2yx2 - 2ryx1(ryx2)(rx1x2)}{\sqrt{1} - r2x1x2}$$

.....Eq.no.1

Data Analysis

The data collected from the study participants was carefully categorized into discrete random variables, primarily focusing on the amount of caffeine intake. Each volunteer's score was derived from their responses to a questionnaire designed to assess symptomatic changes during the caffeine withdrawal period. For statistical purposes, a Multiple Correlation Statistical Method was applied using the formula detailed in Equation No. 1. In this method, two independent variables were utilized: (1) the daily caffeine intake (measured in cups), and (2) the hours of withdrawal. The dependent variable was the severity of insomnia, which was experienced by the participants during the withdrawal period.
The multiple correlation analysis allowed for a comprehensive understanding of the relationship between caffeine intake, the withdrawal period, and insomnia, as reported by the participants. The goal was to determine if higher levels of caffeine intake and longer withdrawal periods correlated with increased insomnia severity.

Result

A total of 68 volunteers were included in the study after meeting the predetermined inclusion and exclusion criteria. All volunteers provided informed consent and underwent a detailed briefing on the study's goals and methodology. The demographic details of the participants are summarized in Table 2. To measure the severity of insomnia before and after the caffeine withdrawal period, the Insomnia Severity Index (ISI) was employed. During the study, it was found that 84% of the participants (59 out of

68) reported difficulty sleeping during the withdrawal phase. This condition met the criteria for substance-induced insomnia, as per the DSM-IV diagnostic manual, demonstrating a notable effect of caffeine withdrawal on sleep patterns. Statistical analysis using the Multiple Correlation Method revealed a mild but statistically significant positive correlation between caffeine intake, withdrawal duration, and insomnia severity. The R-value of 0.22 indicates that while the correlation is not strong, it is still significant enough to suggest that higher caffeine consumption, combined with longer withdrawal, leads to more severe insomnia symptoms.

$$R = \frac{\sqrt{(-0.1751)^2 + (-0.1647)^2 - 2\,(-0.1751)\,(-0.1647)\,(0.1653)}}{\sqrt{1-(0.1653)^2}}$$

$$R = 0.22$$

Discussion

The study comprised 38 females (56%) and 30 males (44%), aged between 18-60 years. According to research by Jeffrey Goldberg, consuming more than 400ml of caffeine daily can lead to several adverse effects, including behavioural changes, insomnia, anxiety, depression, and cardiac arrhythmias. These findings align with our study's observations, where participants consuming 6-8 cups of caffeine per day experienced increased insomnia severity.

The R-value of 0.22 from our correlation analysis supports the hypothesis that there is a positive association between daily caffeine consumption and insomnia during withdrawal. This result underscores the risks of excessive caffeine intake, particularly in relation to sleep disturbances. It also highlights the importance of moderating caffeine intake as a preventive measure for insomnia and other related conditions.

Conclusion

This study demonstrated a significant difference in caffeine intake among rural Indian participants, with higher consumption leading to noticeable effects on sleep. Many participants reported substance-induced insomnia, as defined by the DSM-IV, which emphasizes the need for careful management of daily caffeine intake. The results of the multiple correlation analysis further support the idea that individuals should adjust their caffeine consumption according to their lifestyle and personal tolerance to prevent sleep disturbances. Limitations One of the key limitations of this study is the small sample size (68 participants) and the limited study duration (2 months). To strengthen the findings, future studies should aim to expand the sample size and include participants from urban areas in India to gain a broader epidemiological understanding of caffeine withdrawal and its effects on sleep and behaviour. Further studies could also explore other lifestyle factors that may influence the impact of caffeine on sleep patterns and overall health.

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Author's contribution: Ajith JS and Vijay Bayaskar designed the study and reviewed the manuscript. Mate Vaishnavi and Wagh Bhagyashree written the manuscript, collected the data and did data anaylsis. Matre Vaibhav and Navale Samruddhi were designed the methodology. Supriya Bhagwat , Shubangi Khade , Swati Sandhan, Poonam Wajpei and Mahima Wadje edited the manuscript. On behalf of all the co-authors, I declared that the prepared manuscript is ready for publication.

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Table No.1: Various ingredients present in different amounts of caffeine

Item	Average (mg)	Range (mg)
Coffee (5-oz cup)a		
Brewed, drip method	120	90–150
Percolated	90	64–124
Instant	75	30–120
Decaffeinated	3	1–5
Espresso (6-oz cup)	240	180–300
Teas (loose or bags, 5-oz cup)		
1-minute brew	21	9–33
3-minute brew	33	20–46
Tea products		
Instant (5-oz cup)	20	12–28
Iced (12-oz glass)	29	22–36
Carbonated beverages	24	20–40
Colas and pepper drinks (12 oz)		
National brands, packaged	42	36–48
National brands, fountain	39	32–48
Store brands, packaged	18	5–29
Citrus drinks (12 oz)		
National brands, packaged	52	43–56
Store brands, packaged	38	26–52
Chocolate products		
Cocoa beverage (8 oz)	6	3–32
Chocolate milk beverage (8 oz)	5	2–7

Table No. 2: Mean of the demographic data of volunteers

Criteria	Mean
Gender	Male 51% Female 49%
More than 6 cups of caffeine intake	22.33
BMI	22.98kg/m^2

Immediate Loading with One Piece Implant Following Extraction in Anterior Esthethic Zone

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ABSTRACT

It's understood that one - piece implant design is a stronger conception as there's no connection between implant and abutment. The absence of a microgap can lead to minimum peri- implant bone loss. Likewise, there's a reduction of mechanical complications similar as screw loosening and abutment fractures. These implants can be incontinently placed and can be put through instant function because of their high cortical stabilization. This immediate function protocol has advantages over two - stage surgical placement. Other benefits are smaller surgical movables, reduced treatment time, and minimum trauma. It's suggested that one - piece implant can be a volition to conventional implants for edentulous area where there's a resorbed bone in range and height. Proper treatment planning avoided co-morbidity associated with additional procedures and respectable esthetic result. Simplifying and increasing the effectiveness of treatment and providing greater comfort for the patient with one piece implant compared to conventional two piece implants

KEYWORDS *Clinical Assessment, Piece Dental Implant, Treatment Planning, Esthetics, Provisionals*

INTRODUCTION

Tissue health and implant survival were the main concerns in the early years of contemporary implantology. But in the last few years, there has been a growing understanding that aesthetics is equally as crucial to the end restoration's success as health. Patients are calling for restorations that are both aesthetically pleasing and functional. The doctor faces difficulties when it comes to placing implants and restorations to replace one or more teeth in the aesthetic zone. Indeed, it is a technique sensitive process with minimal margin for error. It is hard and demanding to preserve or develop a soft-tissue scaffold that gives the appearance of a real tooth. Compared to previous generations, single tooth replacements will

probably make up a higher share of prosthetic dentistry in the future. When a posterior tooth is extracted, the dentist may prepare the neighboring tooth, but when an anterior normal looking tooth is to be prepared to serve as a fixed partial denture (FPD) abutment, the patient is more anxious and frequently searches for an alternative. In contrast to missing a posterior tooth, most patients have an emotional reaction to an anterior missing tooth, and financial considerations are less significant. Prospective anterior FPD restorations are never as esthetic as natural teeth because these patients can only see the restorations that appear unnatural. One of the most difficult dental restorations is replacing an anterior tooth. Nevertheless, considering all the benefits of single implant longevity, bone preservation, decreased Single implants are now the preferred treatment for abutment tooth issues and improved neighboring tooth survival. Patient burden would be decreased by placing implants immediately after extraction, provided that the patient is properly examined and Diagnosed. Single stage and quick extraction implants have become more popular in recent years, particularly in the anterior region, where soft-tissue drape is present before the tooth extraction and the patients are more anxious to get a fixed replacement.

Case Report

A chief complaint of 64 year old male patient who came to the prosthetic dentistry department was a movable lower incisors (Figure.1). There are mobile lower incisors present, but #41 absent. The route and range of mandibular motions, the smiling lip line, and the adequate mouth opening were all found during the extraoral clinical examination. There were also no indications of temporomandibular joint dysfunction. Bilateral canine guided occlusion was associated with an intraoral Class I molar relationship. Teeth #31, #32 and #42 were mobile in the patient. When the implant site was examined, the neighboring teeth were found to be vital, normal in color and appearance, and free of any pathological mobility with other teeth. Cone beam computed tomography (CBCT) was done. The patient was informed of the advantages and drawbacks of the following prosthetic treatment choices for replacing the missing incisors: traditional FPD, removable partial denture, and implant-supported prosthesis. The patient chosen a fixed prosthesis supported by implants. On cone beam computed tomography (CBCT) evaluation, the mesiodistal width between #33 and #43 was 16.22 mm [Figure 2]. The buccolingual width with #32 region was 5.3mm, with depth of 11.5mm and buccolingual width with #42 was 5.9 mm with depth of 11.2 mm as per the cross-sectional view in CBCT (Figure 3). The average width of the mandibular incisors is 5.5 mm and 3.5 mm cervically. To accommodate missing incisors in limited space was challenging. The patient insisted for fixed replacement of teeth. All possible treatment options were explained that included teeth supported, resin bonded, as well as Implant-retained Bridge. The consent was taken for the treatment planned. The preference was for two implant with bridge of 4 teeth as the concept of preparation of adjacent teeth was not acceptable. However, space available was not enough for 4 wide two-piece implant which can carry custom abutments supporting 4small crowns. This may be a bad esthetic outcome as compared to two implant supported restorations. To overcome our problem of reduced length and width space for missing incisors, couple of narrow diameter one-piece implants were used for missing incisors (3.75mmx10mm).

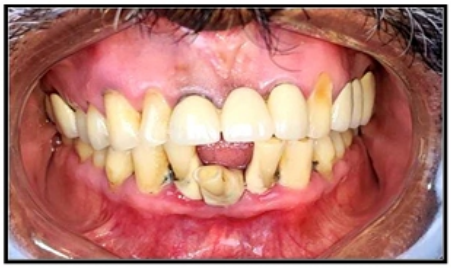

Figure 1: Pre Operative

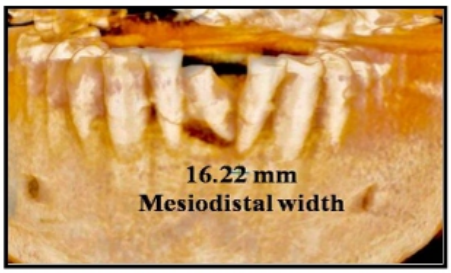

Figure 2: CBCT

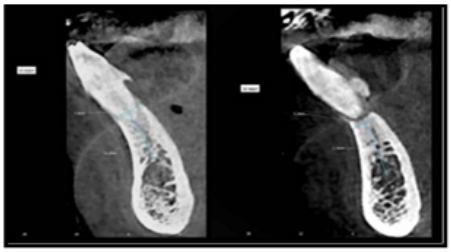

Figure 3: Cross sectional view

Surgical Protocol

Surgical protocol emphasized complete asepsis and infection control. Amoxicillin 1 g for 1 h before surgery. Before the surgical procedure, the patient was instructed to rinse with 0.2%chlorhexidine gluconate for 1 min. The anesthesia (lignocaine 2% with 1:80000 adrenaline, Lignox by Indico remedies ltd., India) was given near the mental foramens bilaterally in the vestibule and infiltration on lingual side. The teeth #31,#32,#42 was extracted. The partial edentulous ridge was exposed with a full thickness mucoperiosteal flap leaving the papilla of the adjacent teeth. The existing edge ridge was slightly flattened with crestal osteotomy. One-piece implant osteotomy is technique sensitive strict manufacturer guidelines should be followed. Initial osteotomy was at 1000 rpm to the required depth (10 mm) with 2mm width drill. The second osteotomy was with 2.8 mm width drill at reduced rpm of 800 rpm for full depth. Third osteotomy drill of 3.2 mm width at 800 rpm was half length of the initial created depth (7 mm). Two one-piece implants (DMi, Israel; 3.75 mm × 10mml) with inter-distance of 5.5mm and 1.5 mm from the adjacent teeth were placed. The torque achieved during insertion was 40Newton. The wound was closed with crossed horizontal sling sutures with nonabsorbable 3-0 Vicryl (Ethicon) (Figure 4). Postoperative IOPA (Figure 5) was taken immediately after surgery. Instructions included soft diet and not to bite from the anterior region for 3 weeks. Oral hygiene was maintained with regular use of fluoride toothpaste except on the surgical area, which was restricted for a week. After 7 days sutures were removed. Prescription included Amoxicillin 500 mg and Ibugesic plus three times daily for 5 days. The patient also used chlorhexidine 0.2% two times daily for 7 days. The implants have an integrated abutment with machined surface for perfect soft tissue bond.

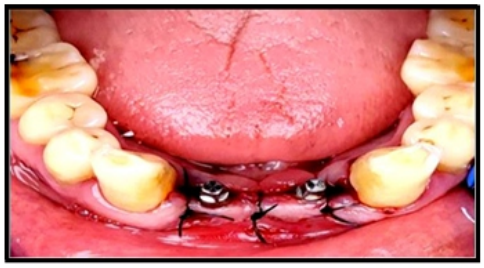

Figure 4: Water tight suturing

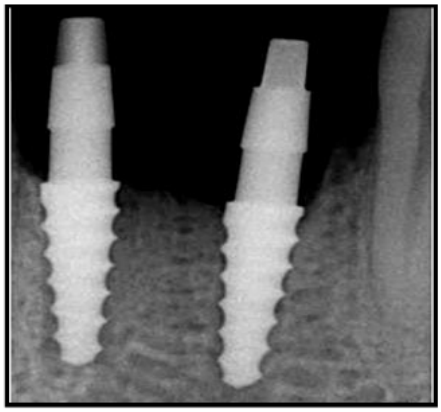

Figure 5: Immediate post operative IOPA

Prosthetic Replacement–Temporary followed by Permanent Prosthesis

Prosthetic Replacement–Temporary followed by Permanent Prosthesis

Four unit temporary acrylic teeth were cemented with provisional cement (TempBond, Kerr Dental) on the abutments on the same day of implant placement (Figure 6). The intaglio surface of the temporary acrylic bridge was egg shaped which may put pressure on the healing tissues for papilla to grow coronally. The occlusion was kept without contact incentric eccentric contacts. After 2months of healing (Figure 7), elastomeric impression with addition silicon and light body (Figure 8) (Hydrorise by zhermack) was taken. Final four unit bridge (IPS e.maxZir CAD) was cemented (Multilink Implant, Ivoclar Vivadent) [Figure 9]. The occlusion was keptwith proper anterior guidance without posterior interferences. Oral hygiene instructions were strictly reinforced. The use of super floss around and beneath the prosthesis was explained. The patient was recalled every 6 months for next 1 year after delivery of prosthesis. At every visit, hard and soft tissue analysis was done.

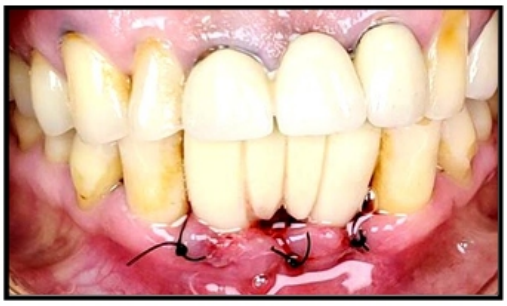

Figure 6: Immediate Temporary Bridge

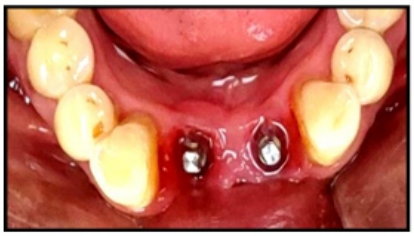

Figure 7: After 2 months

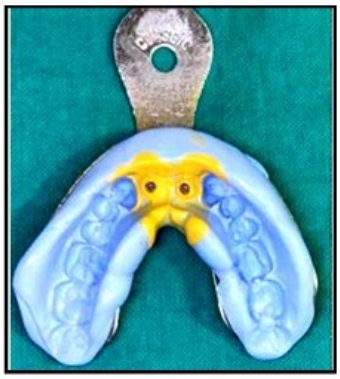

Figure 8: Putty + Light body impression

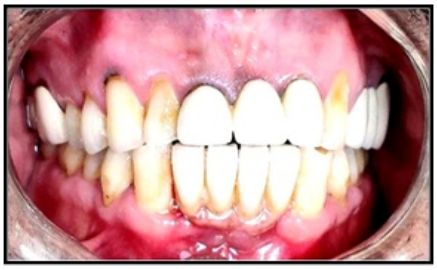

Figure 9: Final prosthesis

Soft Tissue Evaluation

The mean plaque score was better around implant restoration, there was no BOP, the pocket depth was ranging between 0.5 mm, and papillae surrounding the restorations were half the length.

Hard Tissue Evaluation

Radiographs were standardized through paralleling cone technique. The digital caliber measured the space between the bone crest and the fixture at the mesial and distal parts of the one-piece implants in periapical radiographs. The known distance between the two implant threads was used for calibration and determination of the exact magnification of the images. At recall of 1 year, the bone resorption was <0.2 mm from the crestal area [Figures 10 and 11].

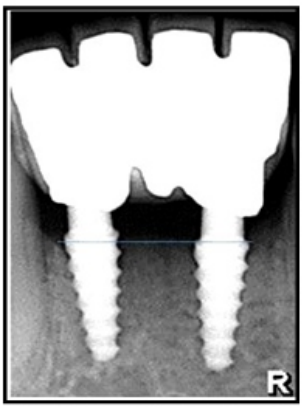

Figure 10: IOPAR with digital caliber measurement

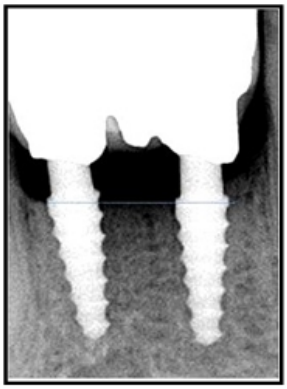

Figure 11: IOPAR After 1 year follow up with Digital caliber measurement

Discussion

A one-piece implant advantages are fast functional, rehabilitation with reduced operating time, less armamentarium, no damage to surrounding tissues, and better use of space limitations. Patient compliance is better with one-piece implants than two stage procedures, less inflammation, pain, and stress because of few prosthetic appointments. Other advantages are better osseointegration, lesser micro movements, and good soft tissue healing. The replacement of mandibular incisors needs special consideration. The challenges associated are limited space, complex surrounding anatomy, and potentially tough esthetic requirements. Missing lower incisors can be rehabilitated with fixed partial dentures, adhesive bridge, or implant retained crowns. In our patient with poor oral hygiene, one tooth was lost and three teeth were extracted but neighboring teeth remain unharmed. Their preparation as abutment teeth would be invasive and may further increase the risk of biological complications such as pulpitis. In gaps with more than one missing tooth, there may be unfavorable physics for a bridge in the anterior zone. A predictable alternative for the replacement of teeth in the said area in some cases is implant retained restoration. Loss of teeth brings resorption and remodeling of surrounding tissues with time. Several approaches like guided bone regeneration with autogenous bone, bone replacement materials in combination with membranes, cortical bone plate method and distraction technique have been described in literature for the defect like ours. A classification of tooth gaps can therefore relate to the bone level of the neighboring teeth and the number of teeth to be replaced: A Class I defect is with loss of a single tooth and a bone level of about 1 mm from the cementoenamel junction of the neighboring teeth, while in a Class II defect, this distance is >1 mm. A Class III defect would have >1 missing tooth. In addition to available bone, there are other anatomical restrictions such as reduced interradicular space for single or multiple implants, proximity of neighboring teeth, and crowding. As the patient was not inclined for extensive augmentation procedures, the treatment done was evidence based and well

accepted by the patient. The current scientific literature supports the concepts that the implants can be loaded early or immediately. Studies regarding different types of prosthesis have shown that early loading of mandibular implants can provide treatment outcomes comparable to those achieved using standard healing periods before loading. The advantages of non-functional immediate teeth are as follows:

1. Patient has a fixed esthetic tooth replacement soon after Stage 1 surgery.
2. No Stage 2 surgery is necessary (eliminates discomfort for the patient and decreases overhead for the doctor)
3. Countersinking the implant below the crestal bone is eliminated, which reduces early crestal bone loss
4. The soft tissue emergence may be developed with the transitional prosthesis and the tissue was allowed to mature during the bone healing process
5. The soft tissue hemi-desmosome attachment on the implant body below the micro gap connection may heal with improved interface.

The disadvantages of non-functional immediate teeth are as follows:

1. Micro movement of implant that can cause crestal bone loss or implant failure is greater than that with two stage surgery
2. The dentist is less likely to reflect the tissue at Stage 2surgery and can evaluate implant crestal bone directly
3. Para function from tongue or foreign habits (pen biting) may cause trauma and crestal bone loss or implant failure.
4. Bone that is too soft, small implant diameters or implant designs with less surface area may cause crestal stress contours and cause bone loss or implant failure.

Conclusion

Nowadays, most people agree that implant therapy is a dependable way to replace lost teeth. However, in order to achieve the best possible aesthetic outcome, the dental implant must be placed correctly during surgery. Determining the ideal implant location and quantity requires careful treatment planning. The time required for soft-tissue healing and implant integration, the development of the emerging profile, occlusal forces in connection to progressive loading, and occlusal forces on the finished restoration are all factors that the physician must take into account.

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Ethnobotanics Study of Medicinal Plants in Lardjem Region (Algeria).

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A B S T R A C T

This study enabled us to make an inventory of the main medicinal plants presents in Lardjem region as well as their therapeutic uses. A series of surveys and field trips allowed us to reveal a multitude of information.

To identify the most used medicinal plants in the study area. Among the most used plants are those belonging to the families Lamiaceae and Liliaceae.

From an ethnobotanical study, young people generally do not know the names or usefulness of most medicinal plants compared to older people. Thus, that there is a large difference in the percentage of plant use by women has a contribution to men. On the other hand the foliage is the most used part and the decoction is the most practiced method. We also note that medicinal plants are widely used to treat diseases of the digestive and respiratory system.

Finally we can conclude that the mode of collection and the high use of certain species are the main factors of degradation plant resources of this region.

KEYWORDS *Medicinal plants Lardjem region - therapeutic uses - families Lamiaceae and Liliaceae - ethnobotanical study - Lardjem – decoction- digestive and respiratory system.*

INTRODUCTION

The use of plants in medicine is very old and usually comes from the belief that they have very low toxicity due to their natural origin. Even wild animals instinctively use certain plants. According to the World Health Organization, around 80% of the world's population uses traditional medicine for health care (Gomes et al. 2012).

Ethnobotanical data estimates that more than 800 plants are used in traditional medicine for treatment (BECHIRI, A, 2016).

Medicinal plants are valuable resources for the majority of rural and urban populations in Africa and represent the main means by which people heal themselves (Badiaga, 2011). Despite progress in pharmacology, the therapeutic use of medicinal plants is very present in some countries of the world and especially in developing countries (Tabuti et al., 2003). Algeria, by the richness and diversity of its flora, constitutes a real phylogenetic reservoir, with about 4000 species and subspecies of vascular plants (Dobignard and Chatelain, 2010-2013). However, the Algerian medicinal flora remains unknown until today, because of the few thousand plant species, only 146 are counted as medicinal (Baba Aissa, 1999).Indeed, traditional medicine has always occupied an important place in the traditions of medication.

The municipality of Lardjem (western Algeria) presents a very important floristic and faunal diversity. For this we deemed it useful to contribute to an ethnobotanical study in the region of Lardjem for the

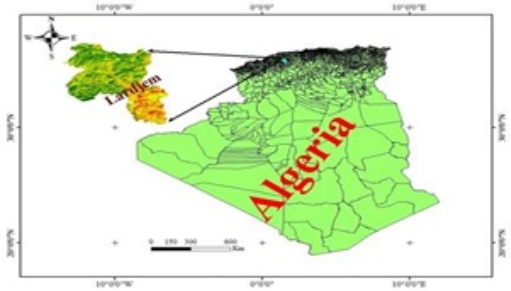
knowledge of medicinal plants, to produce a catalog of these plants in the said region and to gather as much information as possible concerning the therapeutic uses practiced by the local population. Indeed, it is very important to translate this traditional knowledge into knowledge.

Presentation of the study area

Geographical location

This study was conducted in the town of Lardjem west of the wilaya of Tissemsilt, this town covers a total area of 26600 ha. It is located between the coordinates 35° 53' 55", 35° 40' 25" North latitude and 1° 22' 43.24", 1° 36' 33.35" East longitude. It is limited to the north by the municipality of Larbaa, to the south by the municipalities of Sidi Lantri and Maacem, to the east by the municipalities of Bourdj Bounaama, Sidi Abed and Tamellahet, to the west by the wilaya of Relizane and to the south -west by the municipality of Melaab. The forest area in this commune is 17,683.6605 ha, representing an afforestation rate of 66.48%.

Figure №01: Geographical location of the study area.



2. Objectives

For this we deemed it useful to contribute to an ethnobotanical study in the region of Lardjem for the knowledge of medicinal plants, to produce a catalog of these plants in the said region and to gather as much information as possible concerning the therapeutic uses practiced by the local population. Indeed, it is very important to translate this traditional knowledge into knowledge.

3. Methods

Survey and sampling

To highlight the importance of the floristic richness in medicinal plants of the study area and their direction of evolution as well as the main discriminating factors, we started a phytoecological diagnosis, followed by field measurements. Indeed, this simple but effective approach is very necessary to achieve our objectives.

First, we gathered all the cartographic data necessary for carrying out vegetation surveys in the studied area. Several prospecting trips were carried out in order to clarify the value of the sampling plan and concretely locate the transects to be considered. In this phase we carried out floristic surveys at a few stations.

The choice of sites was determined by superimposing three types of information, namely

geomorphology, phytoecology and pedology. The number of stations depends on the change in vegetation cover. In this study we adopted the mixed survey method (linear survey and plot). It turns out to be the most appropriate method for a rapid biological inventory, allowing both to have data on the floristic composition and the structure of the vegetation.

The location of the surveys was determined after a survey. They are carried out in places deemed representative, that is to say places with little disturbance, floristically and physiognomically homogeneous, and according to the topography.

In a second step, we started an ethnobotanical investigation which consists of:

-Collection of medicinal plants from the floristic procession of the main forest formations encountered

- Botanical classification and confirmation of the use of plants in traditional medicine.

-A small survey of herbalists and elderly people who are generally knowledgeable about the use of plants in traditional medicine in the Lardjem region

4. Results

4.1. Ethnobotanical survey:

Through the ethnobotanical survey conducted among the population of the study area, it turns out that there is a diversity of results linked firstly to the variability of the parameters concerning the people surveyed; age group, profession, sex, family situation and level of education. Secondly to the diversity of parameters for the use of medicinal plants, in particular the species used, symptoms treated, parts of the plant used, doses of preparation and method of use. The collected ethnobotanical information was recorded on raw data sheets then transferred to a database, processed and analyzed to obtain standardized data.

4.1.1- Parameters of the respondent:

4.1.1.1- Age group:

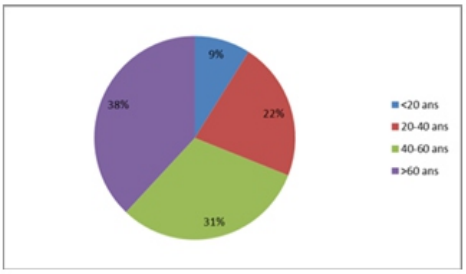


Figure 02: Use of medicinal plants according to the age of respondents.

4.1.1.2- Gender:

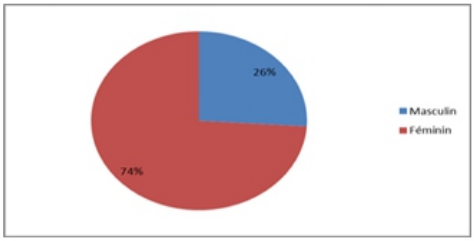


Figure 03: The use of medicinal plants according to the gender of respondents.

4.1.1.3- Academic level:

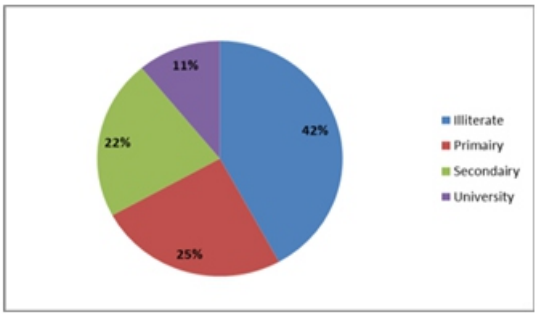


Figure 04: Use of medicinal plants according to the academic level of respondents.

4.1.1.4- Choice between phytotherapy and modern medicine:

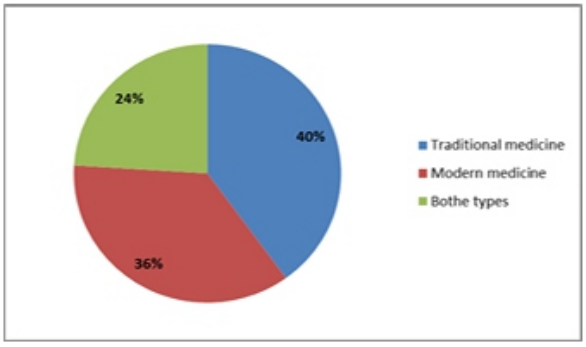


Figure 05: The choice between traditional and modern medicine among people surveyed.

4.1.2- Parameters related to the medicinal plants used:

4.1.2.1- Depending on the species used:

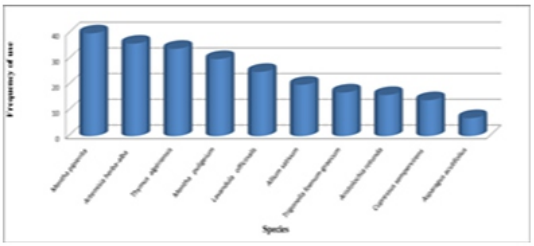


Figure 06: Classification of species according to their frequency of use.

4.1.2.2- According to the type of plant (wild, cultivated):

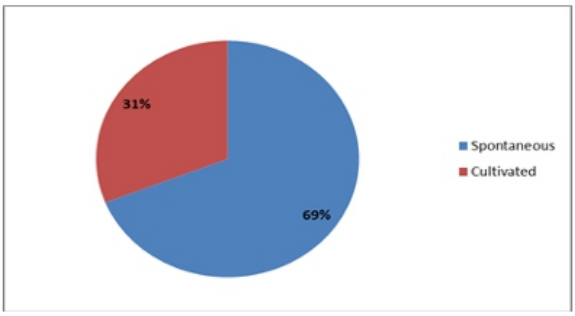


Figure 07: Frequency of use according to the type of plant.

4.1.2.3- Harvest period:

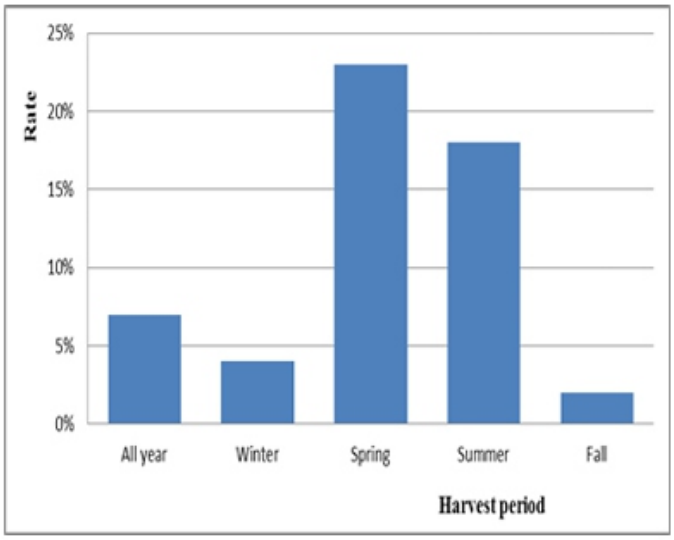


Figure 08: Use of species according to the harvest period.

4.1.2.6- Plant states:

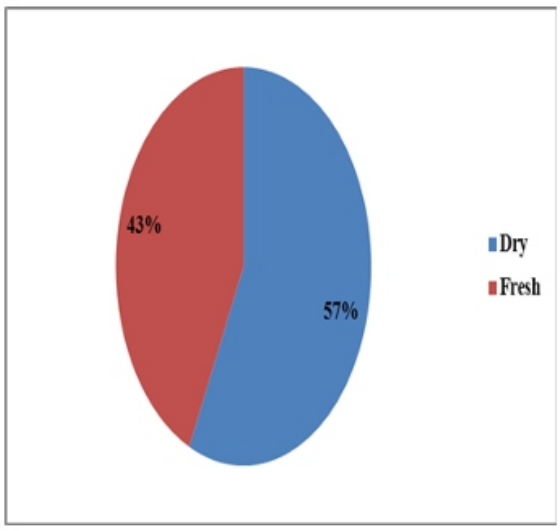


Figure 09: The state of use of medicinal plants.

4.1.2.8- Parts used:

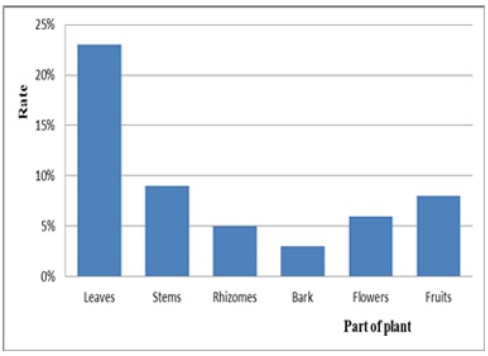


Figure 10: Rate of use of different parts of medicinal plants.

4.2- Diversity of medicinal plants used:

Table 01: Diversity of medicinal plants used.

N=°	Family	Scientific name	Parts used	How to use Indications
1	Liliaceae	*Scille maritime*	plant sap	Digestive disorders, stomach ulcers and inflammation of the intestines.
		Asparagus actufolius	stems, roots	Infections inflammations, minor skin lesions, burns, frostbite and dry skin.
		Allium sativum	Roots	Diuretic, vomiting
		Allium cepa	Roots	Heart fatigue, hypertension, horses digestive system.
2	Ranunculaceae	*Adonis vernalis*	flower petals	The ear, wounds, Digestive system, Respiratory system.
3	Lamiaceae	*Melisse officinalis*	leaves and flowers	Insomnia, excessive nervousness, anxiety, angina and bronchitis. Against inflammation of the eyelids.
		Mentha pulegium	The whole plant	Anemia, difficult digestions, and tuberculosis
		Mentha pipierita	The whole plant	Respiratory disorders, bronchitis and fever.
		Thymus algériensis	Leaves	Digestive tract cough menstruation pain diarrhea.

		Lavandula officinalis	The whole plant	Digestive tract + refreshing + spicy
		Teucrium polium	Leaves	The flu, + rheumatism, + angina
4	Apiaceae	*Thapsia garganica*	Roots and leaves	Chronic eczema, the nervous system, insomnia fevers
5	Fabaceae	*Trigonella foenum graecum*	Seeds	Healing agents + Digestive system
6	Punicaceae	*Punica glanitum*	Flowers, bark, fruits	Used as ear instillation. Fresh leaves, heated and dipped in olive oil, are applied as a poultice to soothe joint pain.
7	Rhamnaceae	*Rhamnus alaternus*	Leaves	Diabetes, stomach pain
8	Cupressaceae	*Juniperus oxycedrus*	Berries, leaves, whole plant	Diarrhea, Digestive System and Asthma
		Cupressus sempervirens	Leaves	Hepatitis + Digestive system
9	Pinaceae	*Pinus halepensis*	Bark, resin, grains	Diabetes- rheumatism- healing (against circumcision)
10	Apocynaceae	*Nerium oleander*	Leaves	Bronchitis + inflammation of the stomach + cough - hair care
11	Tamaricaceae	*Tamarix gallica*	Aerial part	Rheumatism
12	Anacardiaceae	*Pistacia lentiscus*	Leaves	Diabetes, cancer
13	Asteraceae	*Silybum marianum*	Roots	The common cold and gastric hyperacidity
		Artimisia herba-alba	The whole plant	Inflammation of the stomach and colon -diarrhea-healing against burns.
		Chamaemelum nobile	Flowers	Cancer
		Chicoree sauvage	The leaves, roots	gastric pain
14	Cactaceae	*Opuntia ficus indica*	Fruits	migraine and flu + Digestive system.
15	Cistaceae	*Cistus albidus*	Leaves, roots	the urinary tract.
16	Urticaceae	*Urtica dioica*	Leaves,	Digestive

			roots	
17	Boraginaceae	*Borago officinalis*	Roots	Stomach pain- Asthma.
18	Chenopodiaceae	*Spinacia oleracea*	Leaves	Diarrhea +-rheumatism
19	Myrtaceae	*Eucalyptus*	Leaves	Obesity + Respiratory System + Kidneys
20	Thymelaeaceae	*Thymelaea hirsuta L.*	Leaves	Anemia + Digestive System
21	Malvaceae	*Malva sylvestris L.*	The leaves and roots	Diabetes, asthma, Respiratory system, coughs.
22	Poaceae	*Avena sativa*	The grains	hair + dermatoses.
23	Caryophylaceae	*Paronychia argentea*	The leaves and flowers	Eyes, mouth, biting insects

5. Discussion

5.1 Ethnobotanical survey:

5.1.1- Parameters of the respondent:

5.1.1.1- Age group:

The use of medicinal plants in the study area is widespread among all age groups with a predominance of people over 60 years of age (38%). The age groups of 40 to 60 years old, 20 to 40 years old; come next respectively with 31%, 22%. However, people in the age group under 20 (9%) do not use traditional medicine much for their medical safety. These values confirm the results obtained in other studies on the use of medicinal plants.

The elderly are familiar with traditional herbal medicine compared to other age groups, similarly, the lack of interest in herbal medicine among people of this age group is explained by the mistrust, particularly of young people who tend to no longer believe too much in traditional medicine.

5.1.1.2- Gender:

female sex predominates with a percentage of 74%. Moreover, in the male sex this rate does

In the study area, both women and men practice traditional medicine. However, the

This is explained by the unawareness of illiterate people of the dangers caused by the irrational use of medicinal plants, other illiterates cannot precisely understand theverbal instructions transmitted by herbalists and healers. This high rate of illiterate users of medicinal plants is a real obstacle to local development.

5.1.1.3- Academic level:

Out of all respondents, illiterates dominate with a rate of (42%). While people with only one of the primary and secondary academic levels reach rates of (25%) and (22%) respectively. Finally, among those at the university level, the rate is 11%. not exceed 26%. This explains why women are more concerned with phytotherapeutic treatment and the preparation of plantbased recipes, not only for

the whole family. As he shows us that woman are more holders of traditional phytotherapeutic knowledge than men.

5.1.1.4- Choice between phytotherapy and modern medicine:

Concerning the choice of people surveyed between therapeutic practices in the study area, the results obtained show that 40% of the population use traditional medicine, while 36% prefer modern medicine and a rate of 24% of people use two at a time. This is justified by the fact that the local population is interested in traditional remedies to relieve their daily ailments.

5.1.2- Parameters related to the medicinal plants used:

5.1.2.1- Depending on the species used:

According to the results obtained from the ethnobotanical survey carried out in the study area, a list of species most used in traditional medicine and their frequency of use by the population surveyed (Fig.06) was found. results showed that among the 10 most used species, the species Mentha pipierita is first followed by the two species (Artemisia herba–alba Thymus algériensis), then come the other species such as Mentha puligeium, Lavandula officinalis, Allium sativum, Trigonella foenum-graecum, Aristolochia rotunda, Cupressus sempervirens according to their degree of use and lastly we have the species Asparagus acutifolius with a low frequency of use.

5.1.2.2- According to the type of plant (wild, cultivated):

The ethnobotanical survey shows us that spontaneous plants are widely used with 69% of the total species. This is due to their year-round availability. Unlike the cultivated ones which only reach utilization rate of around 31%.

5.1.2.3- Harvest period:

The results of our survey in the study area show that the harvest of certain species usable in traditional medicine can be throughout the year while for others is distributed according to the seasons, noting that spring and summer come at first place while winter and fall record minority harvest rates.

5.1.2.6- Plant states:

According to the results obtained, it was found that 57% of the medicinal species are used in the dry state; they constitute the basis of herbal teas, powders and extracts usable in traditional medicine. On the other hand, 43% of the species are used fresh; they are mainly used in the preparation of mother tinctures, poultices and soups.

5.1.2.8- Parts used:

The results of our ethnobotanical survey revealed that the foliage is the most used part in the study area with a rate of (23%), followed by stem (09%), then the fruits (08%), the flowers (06%), while the rhizomes and the other parts of the plants (twig, bud, bark) are used with low rates respectively (05%) and (03%).

This difference in proportions in the used parts of the plant is justified by the variability of concentration of active substances in each organ. The dominance of the leaves is justified by the fact that they are the site of the majority of the photochemical reactions and considered as a reservoir of the organic matter that derives from them. The leaves provide the majority of the alkaloids, glycosides and essential oils.

The importance of fruits is due to the concentrations of their substances carbohydrates and aromatics associated with certain pigments which give them a characteristic coloring. The use of flowers is due to their richness in oil Essential. The same is true for roots and seeds rich in sugars and vitamins.

The harvesting of these organs is done in an arbitrary way by the local population who ignores the phenological phases, leafing, flowering and fruiting, which consequently exerts a strong pressure of harvesting leading to the decrease in productivity, reduction and loss of the biodiversity. This way of harvesting leads to the rarefaction, or even the risk of the total disappearance of certain species.

5.2- Diversity of medicinal plants used:

The results obtained are listed in Table01. according to therapeutic practices, use of plants as well as the treatment of diseases. All the medicinal species identified are represented in the form of a catalog.

The analysis of the results was based on the vernacular name of the plants; all the plants thus identified are well known in the traditional Algerian pharmacopoeia and are used in the region of Lardjem for therapeutic purposes.

Conclusion

Despite the development of the drug industry of chemical origin, traditional herbal medicine is currently a source of remedy par excellence. The latter is widely distributed among populations who trust popular medical use and do not have the means to bear the costs of modern medicine. Indeed, phytotherapy plays a very important role in the modern therapeutic field, by constituting a database through ethnobotanical study.

The latter is rich in empirical knowledge resulting from the experiences of men.

Thus, the present study has made it possible to carry out the most complete inventory possible of the medicinal plants used in an area which is part of the Aurès region of Algeria and to gather information concerning the therapeutic uses practiced in this area.

The ethnobotanical survey revealed a multitude of results. About 35 medicinal plant species are reported, divided into 23 families, of which the most used species is the Mentha piperita, and the most represented family is Lamiaceae.

The people surveyed are mostly over 60 year's old, illiterate, married and have no profession. Also, medicinal plants attract much more attention from women who know their value and therapeutic effects better than men.

The use of spontaneous medicinal plants dominates that of cultivated plants and most of these plants are harvested manually, especially in spring. In addition, the majority of medicinal plants are used alone without association with other plants, generally in a dry state.

The leaves are the most used part and most recipes are prepared mainly with non-specific doses in the form of an infusion. These doses vary according to age, of which the daily dosage for adults is the most

numerous.

These plant-based recipes are administered orally, especially in the form of herbal tea. Thus, the distribution of the frequency of use of medicinal plants according to the group of diseases treated shows that digestive disorders are the major therapeutic indications. The most commonly used duration treatment corresponds to a week. The use of herbal medicine is not devoid of certain risks due to the toxicity of certain plants, which requires taking precautions for use. Similarly, the collection and analysis of the data collected have made it possible to transform popular oral knowledge in this region into transcribed knowledge by establishing a catalog of the medicinal plants used and their therapeutic use.

Indeed, it is necessary and important to safeguard the phytotherapeutic knowledge of the Aurès population in Algeria because they are part of the national heritage which deserves to be valued.

Moreover, these results can be considered as a source of information for scientific research in the field of phytochemistry and pharmacology with a view to finding new active principles based on plants.

Medicinal plants still remain the reliable source of active ingredients known for their therapeutic properties.

As well as they must have, like medicines, strict standard rules that only the specialist in herbal medicine can meet.

Finally, more importance must be given to the cultivation, exploitation and marketing of these plants, which can be an important source of external income.

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Synthesis and Biological Activity of Novel Imidazole Based Chalcone Derivatives.

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ABSTRACT

***Aim**: To synthesize novel imidazole-based chalcone derivatives and assess their biological activities for potential drug development.*

***Method:** The novel imidazole-based chalcone derivatives were synthesized by reacting benzylamine, dihydroxyacetone, and potassium thiocyanate, forming (1-benzyl-2-mercapto-1H-imidazol-5-yl) methanol. The introduction of methyl or ethyl iodide, coupled with oxidation using magnesium dioxide, yielded the target compounds by alkylation and oxidation method. Claisen-Schmidt condensation with various acetophenones under methanolic sodium hydroxide facilitated further derivatization. Structural elucidation involved Spectroscopic analysis and mass spectrometry, followed by antimicrobial activity testing.*

***Result**s: Compounds (9a-9j') demonstrated varied antibacterial efficacy. Compound 9b exhibited notable activity against B. cereus and E. coli (MIC 125 µg/mL and 62.5 µg/mL). Compound 9e showed significant antibacterial activity across strains (MIC 50-125 µg/mL), and 9c consistently inhibited S. aureus, B. cereus, and P. aeruginosa (MIC 250 µg/mL). Compounds 9g and 9j' displayed diverse antibacterial effects, indicating potential selectivity.*

Conclusions: In conclusion, the synthesized imidazole-based chalcone derivatives exhibit promising antimicrobial potential, supported by methodological alignment and consistent observations in antibacterial and antifungal activities. The innovative inclusion of oxidation steps enhances structural diversity, emphasizing their efficacy against bacterial and fungal infections.

***KEYWORDS** Antimicrobial activity, Chalcones, Claisen-Schmidt, Imidazole, Nuclear magnetic resonance*

INTRODUCTION

The prevalence of azoles in both natural and synthetic compounds, coupled with their pivotal role as synthetic has attracted significant attention across industrial and academic domains [1-5]. Azole-based heterocyclic derivatives, renowned for their diverse biological activities and therapeutic potential, have emerged as focal points in medicinal chemistry research [6-10]. The azole scaffold is a crucial element found in many medications that have been authorized for clinical use and in compounds that are being investigated, highlighting its significance in contemporary drug development endeavours [11-13]. Azole ring-containing compounds, such as imidazole, triazoles, and oxazole, have shown significant efficacy against a range of ailments, including microbial infections, cancer, inflammation, and

neurological problems [14,15]. Azole derivatives, including triazoles and imidazole, are extensively used as antifungal medicines because they may effectively hinder fungal cytochrome P450 enzymes that play a vital role in the production of ergosterol, a critical element of fungal cell membranes [16-19]. Imidazole-based chalcone derivatives represent a class of compounds with promising potential in medicinal chemistry due to their diverse biological activities [2022]. Chalcones, characterized by their α, β-unsaturated ketone structure, have long been recognized for their pharmacological properties. Imidazole, on the other hand, serves as a privileged scaffold in drug discovery owing to its structural versatility and pharmacological relevance [23-27]. The fusion of imidazole with chalcone moieties presents an intriguing avenue for the synthesis of novel compounds with enhanced biological profiles.

Several studies have underscored the pharmacological significance of imidazole-based compounds. For instance, research by Sharma et al., (2021) demonstrated the anticancer potential of imidazole derivatives against various cancer cell lines, highlighting their role as cytotoxic agents [28]. Additionally, investigations by Patel et al., (2021) revealed the antimicrobial activity of imidazole-based compounds against pathogenic microorganisms, suggesting their utility as antimicrobial agents [29].

Moreover, the biological activities of chalcone derivatives have been extensively explored in the literature. Studies by Li et al., (2020) elucidated the antioxidant properties of chalcones, emphasizing their potential in combating oxidative stress-related diseases [30]. Furthermore, research conducted by Rashid et al., (2019) highlighted the anti-inflammatory effects of chalcone derivatives, indicating their therapeutic relevance in inflammatory conditions [31].

In this perspective, the synthesis and biological evaluation of novel imidazole-based chalcone derivatives represent an important and fascinating area of study. Through the integration of the structural characteristics and pharmacological attributes of imidazole and chalcone, these compounds have the potential for the creation of highly effective therapeutic agents that may target a wide range of disorders, such as cancer, microbial infections, oxidative stress, and inflammation [32-36].

This research paper aims to explore the synthesis strategies employed for the preparation of imidazolebased chalcone derivatives and investigate their biological activities through comprehensive in vitro and in vivo evaluations. Through this endeavour, insights into the pharmacological potential of these compounds can be gained, paving the way for their future development as clinically relevant therapeutics.

2. Methods

2.1 Materials

Benzyl amine, dihydroxyacetone, potassium thiocyanate, acetic acid, 1-butanol, methyl iodide or ethyl iodide, sodium hydroxide, magnesium dioxide (MnO2), chloroform, acetophenones, methanolic sodium hydroxide, oxone, tetrahydrofuran (THF) and water were the reagent used in the synthesis of compounds. The chemicals and reagents were procured from Sigma Aldrich.

2.2 Synthesis Derivatives of Imidazole-Based Chalcone

The synthesis of imidazole-based chalcone derivatives, namely compounds 7a and 7b, was carried out using a multi-step approach described in Scheme 1. To, a solution of acetic acid and 1-butanol benzylamine (1), dihydroxyacetone (2), and potassium thiocyanate (3) were mixed. The reaction mixture after reaction, containing (1-Benzyl-2-mercapto-1H-imidazol-5-yl) methanol was agitated for

72 hours. Later, a solution of N-(4-methylphenyl)-2-aminothiocarbonylpropanoic acid (compound (4)) in methanol was treated with methyl iodide ethyl iodide and then coupled with an aqueous solution of sodium hydroxide. This led to the synthesis of 2-(4-methylphenyl)-4-thioxo-4,5-dihydro-1Himidazole-5-carboxylic acid and 2-(4-methylphenyl)-4thioxo-1H-imidazole-5-carbaldehyde (compounds 5, 6). Subsequently, compounds 5 and 6 were oxidized using magnesium dioxide (MnO2) under reflux conditions with chloroform as a solvent, resulting in the formation of 2(4-methylphenyl)-1H-imidazole-4-thiol (compound 7). Subsequently, Claisen-Schmidt condensation processes were conducted using compound 7a or 7b with different acetophenones (8a-8j) in presence of methanolic sodium hydroxide solution resulting in the formation of compounds 9a-9j'. In addition, compound 9j was oxidised using Oxone in a mixture of THF and water, forming the oxidized methyl sulfonyl molecule 9k. The chemical integrity and content of all synthesized compounds were verified using nuclear magnetic resonance (1H NMR, 13C NMR) and mass spectrometry studies, confirming their structural properties. The use of this complete synthetic strategy facilitated the production of imidazole based chalcone derivatives having welldefined structures and potential biological activity.

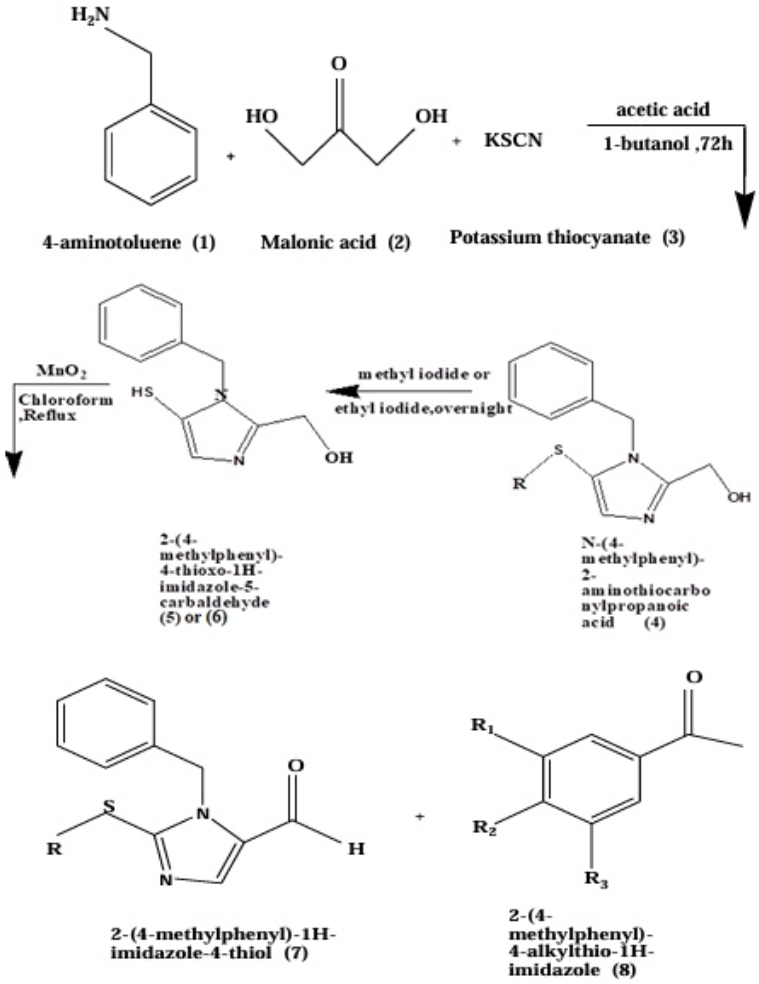


Figure 1: Scheme 1- Reagents and conditions.

Table 1. - Compounds 7a-9j				
Compounds	**R_1**	**R_2**	**R_3**	**R_4**
7a	H	H	H	Me
7b	H	H	H	Et
8a	H	Br	H	-
8b	H	F	H	-
8c	H	Cl	H	-
8d	H	NO_2	H	-
8e	H	OPh	H	-
8f	H	CH_3	H	-
8g	Ph	Ph	H	-
8h	H	Ph	H	-
8i	H	OCH_3	H	-
8j	OCH_3	OCH_3	OCH_3	-
9a	H	Br	H	SMe
9b	H	F	H	SMe
9c	H	Cl	H	SMe
9d	H	NO_2	H	SMe
9e	H	OPh	H	SMe
9f	H	CH_3	H	SMe
9g	Ph	Ph	H	SMe
9i	H	OCH_3	H	SMe
9j	OCH_3	OCH_3	OCH_3	SMe
9K	OCH_3	OCH_3	OCH_3	SO_2Me
9a'	H	Br	H	SEt
9b'	H	F	H	SEt
9c'	H	Cl	H	SEt
9e'	H	OPh	H	SEt
9g'	Ph	Ph	H	SEt
9h'	H	Ph	H	SEt
9i'	H	OCH_3	H	SEt
9j'	OCH_3	OCH_3	OCH_3	Set

2.3 Characterization

The synthesized novel imidazole-based chalcone derivatives were spectroscopically characterized using advanced analytical instruments. Mass spectrometry was conducted using a Bruker MicroTOF-Q mass spectrometer, sourced from Bruker India Scientific Pvt. Ltd., to determine the molecular weights and structural properties of the compounds. Nuclear magnetic resonance (NMR) spectroscopy, including both carbon (13C) and proton (1H) NMR, was performed using a Bruker AVANCE III 500 MHz NMR spectrometer, also supplied by Bruker India Scientific Pvt. Ltd. These instruments provided high-resolution spectra, enabling precise determination of the molecular structures of the synthesized chalcone derivatives.

2.5 Statistical Analysis

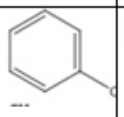
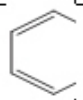
The statistical tests were run using the SPSS (Version 26) programme. While numbers and percentages were utilised to depict categorical data, the mean and standard deviation were used to characterise all continuous variables. Descriptive statistics were used to determine the frequencies and proportions.

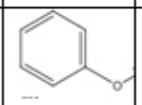
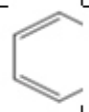
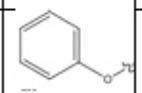
3. Results

The different Chalcone Derivatives were synthesized using Claisen-Schmidt condensation reaction. This involves potent bases like NaOH or KOH in polar solvents such as MeOH or DMF. Thin-layer chromatography confirms the purity of compound, and spectrum analysis confirm the structured analysis. Table 2 below displays the physical data of different imidazole triazole -chalcones derivatives.

Table 2: Physical data of synthesized imidazothiazole-chalcones derivatives (9a-9j')

Compounds	R_1	R_2	R_3	R_4	M.P. (°C)	Yield (%)

9a	H	Br	H	SCH_3	237-239	62.27
9b	H	F	H	SCH_3	287-290	66.11
9c	H	Cl	H	SCH_3	271-274	52.85
9d	H	N_2O	H	SCH_3	251-253	50.02
9e	H		H	SCH_3	280-281	61.98
9f	H	CH_3	H	SCH_3	267-269	63.79
9g			H	SCH_3	246-249	58.28
9i	H	OCH_3	H	SCH_3	249-250	67.49
9j	OCH_3	OCH_3	OCH_3	SCH_3	282-284	68.14
9k	OCH_3	OCH_3	OCH_3	SO_2CH_3	279-280	64.58
9a'	H	Br	H	S-Ethyl	222-228	61.78
9b'	H	F	H	S-Ethyl	262-	76.87

					265	
9c'	H	Cl	H	S-Ethyl	272-274	50.89
9e'	H		H	S-Ethyl	252-258	78.67
9g'			H	S-Ethyl	276-280	45.56
9h'	H		H	S-Ethyl	212-218	78.45
9i'	H	OCH_3	H	S-Ethyl	247-250	32.98
9j'	OCH_3	OCH_3	OCH_3	S-Ethyl	222-228	58.89

3.1 Synthesis (a)3-(1-benzyl-2-(methylthio orethylthio)-1Himidazol-4-yl)-1-phenylpropan-1-one (9a-9j')

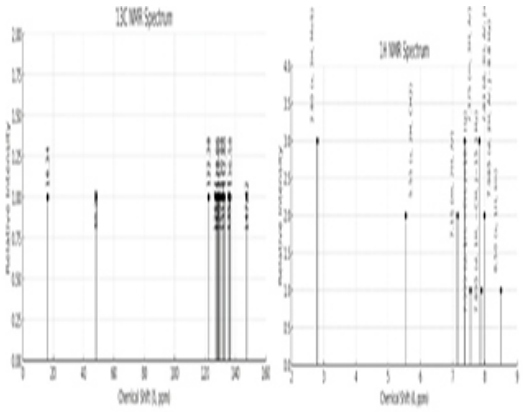
A solution containing sodium hydroxide (2 moles/litre), consisting of 2 ml, was combined with either 1-benzyl2-mercapto-1H-imidazole-5-carbaldehyde derivative 7a or 7b, with a quantity of 1.0 ml, in a solution of methanol with a volume of 5.0 ml. Subsequently, the appropriate acetophenone, specifically 8a-8j, with a quantity of 1 mmol, was added to the mixture. The resulting mixture was stirred at room temperature for a duration of 24 to 48 hours, with the progress of the reaction monitored using thin-layer chromatography (TLC). Following completion, 2 N hydrochloric acid was added until a solid was produced. After filtering and rinsing with cold ethanol, the raw material was refined by recrystallization in methanol.

• (E)-3-(1-benzyl-2-(methylthio)-1H

imidazole-4-yl)-1-(4-bromophenyl) prop-2-en-1-one (9a)

Yield :62% melting point = 202–204 °C. The 1H NMR 200 MHz DMSO-d6 (δ) in ppm: 2.80 (singlet, 3H, methylthio group), 5.55 (singlet, 2H, methylene group), 7.14–7.17 (multiplet, 2H, aromatic protons), 7.32–7.43 (multiplet, 3H, aromatic protons), 7.53–7.58 (doublet, 1H, vinyl proton, J = 18.7 Hz), 7.76–7.90 (doublet, 3H, 4-bromophenyl protons H4 and H6, J = 7.8 Hz), 7.877.92 (doublet, 1H, vinyl proton, J = 15.3 Hz), 7.97–8.00 (doublet, 2H, 4-bromophenyl protons H3 and H5, J = 8.8 Hz), 8.50

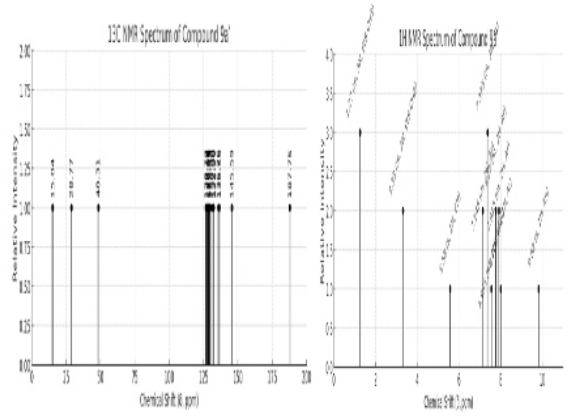
(singlet, 1H, imidazole proton). 13C NMR spectra at 75 MHz in DMSO-d6 (δ) at 16.34, 48.23, 122.381, 126.87, 128.06, 128.55, 128.60, 129.52, 130.88, 131.88, 132.8, 135.63, 136.58, and 147.42. The LC-MS: m/z 414.2 (M + H) +.



Compound (9a)

• (E)-3-(1-benzyl-2-(ethylthio)-1H-imidazol-4yl)-1-(4-bromophenyl) prop-2-en-1-one (9a')

Yield :74% melting point =154–156 ◦C. The 1H NMR δ (ppm) 1.24–1.29, 3.29–3.36, 5.58, 7.13–7.16, 7.32–7.43, 7.53–7.58, 7.75–7.79, 7.89–7.94, 7.99–8.02, and 9.82. The 13C NMR analysis δ (ppm) 15.04, 28.77, 48.31, 126.77, 127.19, 128.06, 128.51, 128.64, 129.49, 130.89, 132.00, 132.36, 135.76, 136.55, 145.59, and 187.75. The LC-MS :427.3 (M + H) +.

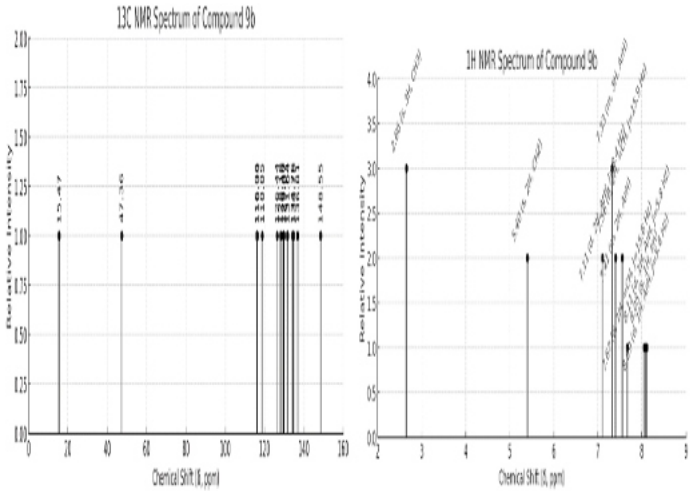


Compound (9a')

(E)-3-(1-benzyl-2-(methylthio)-1H-imidazol 4-yl)-1-(4- fluorophenyl) prop-2-en-1-one (9b)

Yield: 42%, melting point =182 -184 °C. The 1H NMR (300 MHz, DMSO-d6) at δ (ppm): 2.65-2.66 (singlet, 3H, CH3, J = 4.6 Hz), 5.40 (singlet, 2H, CH2), 7.09-7.12 (doublet, 2H, ArH, J = 8.4 Hz), 7.28-7.37 (multiplet, 3H, ArH), 7.39-7.42 (multiplet, 2H, ArH), 7.53-7.58 (doublet, 2H, HC = CH, J = 15.9Hz), 7.64-7.70 (doublet, 1H, HC = CH, J = 15.6 Hz), 8.05 (singlet, 1H, imidazole), 8.08-8.10 (doublet, 1H, ArH, J = 5.4 Hz), 8.10-8.12 (doublet, 1H, ArH, J = 5.8 Hz). 13C NMR spectrum (80 Mhz,

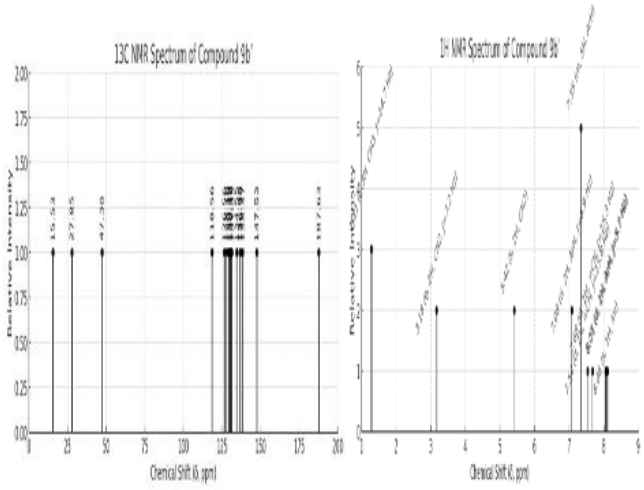
DMSO-d6) displays signals at the following chemical shifts (δ in ppm): 15.47, 47.36, 116.06 (CF), 116.34 (CF), 118.85, 126.44, 128.18, 129.40, 129.87, 131.54, 131.64, 134.26, 134.78, 136.81, 148.55. Analysed by LC-MS (ESI), peaks observed at 353.4 (M + H) + and 375.5 (M + Na) +.



Compound (9b)

• (E)-3-(1-benzyl-2-(ethylthio)-1H-imidazol-4yl)-1-(4-fluorophenyl) prop-2-en-1-one (9b')

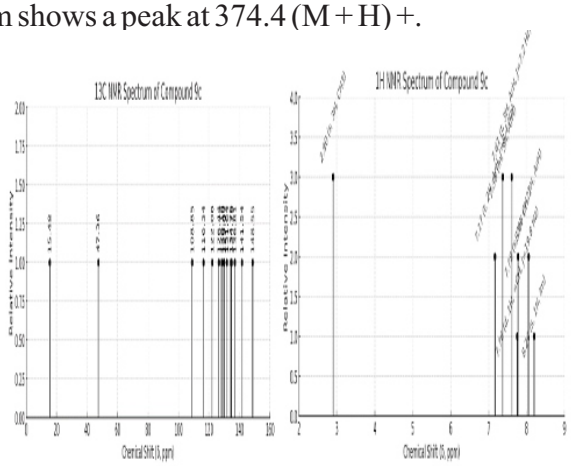
Yield: 41%., Melting point =134-136°C. 1H NMR spectrum (300 MHz-DMSO d6) at δ (ppm) 1.27-1.31 (triplet, 3H, CH3, J = 14.7 Hz), 3.12-3.23 (quartet, 2H, CH2, J = 7.2), 5.42 (singlet, 2H, CH2), 7.07-7.09 (doublet, 2H, ArH, J = 6.9 Hz), 7.28-7.41 (multiplet, 5H, ArH), 7.51-7.56 (doublet, 1H, HC = CH, J = 15.8 Hz), 7.65-7.70 (doublet, 1H, HC = CH, J = 15.3 Hz), 8.06 (singlet, 1H, imidazole), 8.07-8.09 (doublet, 1H, ArH, J = 5.7 Hz), and 8.10-8.12 (doublet, 1H, ArH, J = 5.7 Hz). The compound's elemental analysis data was as follows: 15.53, 27.85, 47.38, 118.56, 126.56, 128.16, 129.39, 130.17, 130.58, 131.39, 134.55, 136.73, 136.90, 138.37, 147.53, and 187.63. LC-MS (ESI) analysis at 367.4 (M + H) +.



Compound (9b')

• (E)-3-(1-benzyl-2-(methylthio)-1H-imidazol4-yl)-1-(4- chlorophenyl) prop-2-en-1-one (9c)

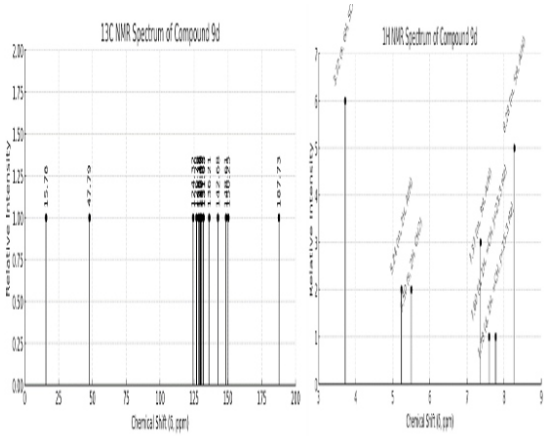
Yield: 59%, Melting point =183-185 °C. The 1H NMR spectrum (250 MHz, DMSO-d6) displays peaks at δ (ppm): 2.90 (singlet, 3H, CH3), 7.75-7.80 (singlet, 2H, Ch2), 7.14-7.19 (triplet, 2H, ArH), 7.29-7.46 (multiplet, 3H, ArH), 7.56-7.66 (triplet, 3H, ArH), 7.72-7.79 (doublet, 1H, HC = CH, J = 19.8 Hz), 8.03-8.08 (multiplet, 2H, ArH), and 8.20 (singlet, 1H, imidazole). The 13C NMR spectrum (72 MHz, DMSO-d6) shows peaks at the following chemical shifts (δ in ppm): 15.48, 47.36, 122.06 (CF), 116.34 (CF), 108.85, 126.44, 128.18, 129.40, 129.87, 141.54, 131.64, 134.26, 134.78, 136.81, 148.55. The compound's LCMS (ESI) spectrum shows a peak at 374.4 (M + H) +.



Compound **(9c)**

• (E)-3-(1-benzyl-2-(methylthio)-1H-imidazol 4-yl)-1-(4- nitrophenyl) prop-2-en-1-one (9d)

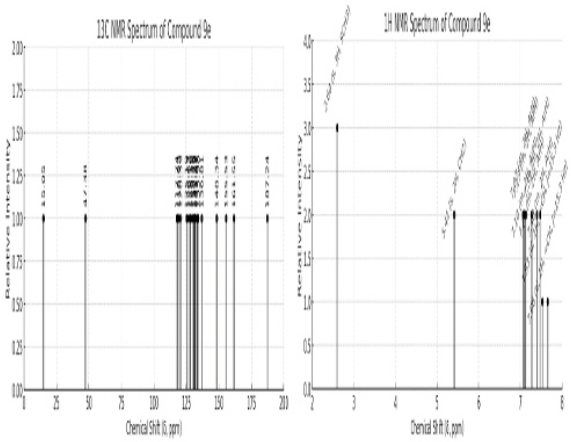
Yield : 12%; Melting point = 166–169 ◦C; 1H NMR (300 MHz-DMSO d6): The chemical shifts (δ) in parts per million (ppm) are as follows: 3.72 (singlet, 6H, SCH3), 5.50 (singlet, 2H, CH2), 5.17–5.30 (multiplet, 2H, ArH), 7.30–7.43 (multiplet, 3H, ArH), 7.57–7.62 (doublet, 1H, CH = CH, J = 23.3 Hz), 7.77–7.78 (doublet, 1H, CH = CH, J = 15.3 Hz), 8.21–8.34 (multiplet, 5H, ArH). 13C NMR (75 MHz DMSO d6): The chemical shifts (δ) in parts per million (ppm) are as follows: 15.78, 47.79, 129.14, 124.32, 126.78, 128.38, 129.47, 130.09, 130.35, 131.63, 131.95, 136.21, 142.68, 148.61, 150.25, 187.73. LC-MS (ESI): (M + H) + ratio of 380.4.



Compound (9d)

• (E)-3-(1-benzyl-2-(methylthio)-1H-imidazol 4-yl)-1-(4- phenoxy phenyl) prop-2-en-1-one (9e)

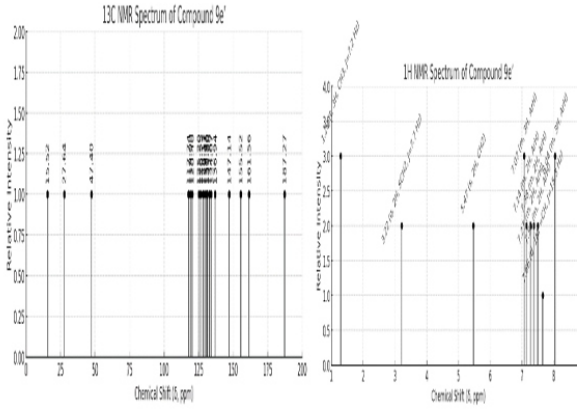
Yield: 46%; Melting point = 144–146 oC ; 1H NMR spectrum (200 MHz-DMSO d6) as shown in Figure 2: δ (ppm) 2.60 (singlet, 3H, SCH3), 5.41 (singlet, 2H, CH2), 7.05–7.08 (multiplet, 2H, ArH), 7.08–7.11 (multiplet, 2H, ArH), 7.11–7.15 (multiplet, 2H, ArH), 7.23–7.31 (multiplet, 2H, ArH), 7.37–7.42 (multiplet, 2H, ArH), 7.44–7.51 (multiplet, 2H ArH), 7.51–7.54 (doublet, 1H, CH = CH, J = 15.3 Hz), 7.63–7.68 (doublet, 1H, CH = CH, J = 15.3 Hz);13C NMR spectrum (75 MHz-DMSO d6): δ (ppm) 15.05, 47.38, 117.74, 118.83, 120.39, 125.19, 126.63, 128.16, 129.38, 130.79, 131.25, 131.58, 132.91, 133.98, 136.81, 148.34, 155.53, 161.55, 187.24; LC-MS (ESI): 427.3 (M + Na)+.



Compound (9e)

• (E)-3-(1-benzyl-2-(ethylthio)-1H-imidazol-4 yl)-1-(4- phenoxy phenyl) prop-2-en-1-one (9e')

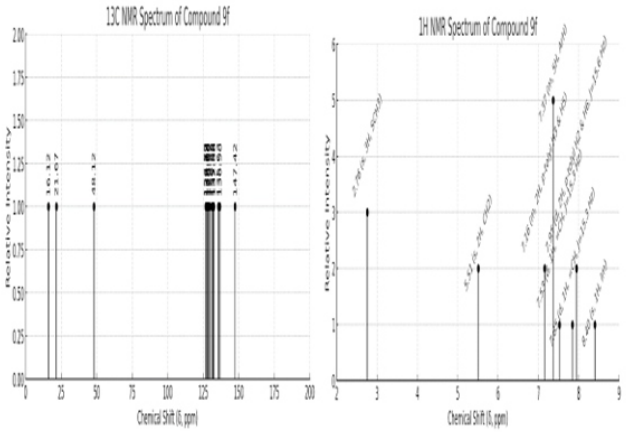
Yield:41%. The melting point =129 -132°C. 1H NMR spectrum (300 MHz, DMSO-d6) displays increases at δ 1.27-1.31 (triplet, 3H, CH3, J = 7.2 Hz), 3.16-3.27 (quartet, 2H, SCH2, J = 7.7 Hz), 5.47 (singlet, 2H, CH2), 7.05-7.09 (multiplet, 3H, ArH), 7.13-7.15 (multiplet, 2H, ArH), 7.23-7.31 (multiplet, 2H, ArH), 7.35-7.40 (multiplet, 2H, ArH), 7.45-7.55 (multiplet, 2H, ArH), 7.63-7.68 (doublet, 1H, CH = CH, J = 15.3 Hz), and 8.038.05 (multiplet, 3H, ArH). 13C NMR spectra (75 MHz, DMSO-d6) display peaks at δ 15.52, 27.64, 47.40, 117.74, 119.00, 120.40, 125.20, 126.55, 128.13, 129.37, 129.41, 130.80, 131.26, 132.89, 134.07, 136.94, 147.14, 155.52, 161.56, and 187.27. The compound's molecular weight was found to be 441.6 (M + 1) + and 463.3 (M + Na) + using LC-MS (ESI).



Compound (9e')

• (E)-3-(1-benzyl-2-(methylthio)-1H-imidazol 4-yl)-1-(p-tolyl) prop2-en-1-one (9f)

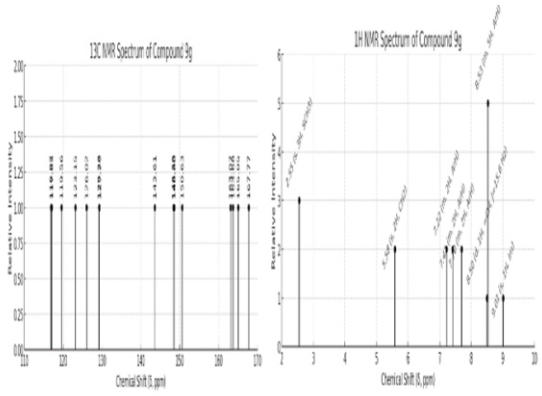
Yield: 81%; Melting point =116–124 ◦C; 1H NMR spectrum (300 MHz, DMSO-d6) displays increases at δ 2.39-2.76 (triplet, 3H, SCH3, J = 7.2 Hz), 3.16-3.27 (quartet, 2H, SCH2, J = 7.7 Hz), 5.51 (singlet, 2H, CH2), 7.14-7.17 (multiplet, 3H, ArH), 7.13-7.15 (multiplet, 2H, ArH), 7.30-7.43 (multiplet, 2H, ArH), 7.35-7.40 (multiplet, 2H, ArH), 7.45-7.55 (multiplet, 2H, ArH), 7.50-7.55 (doublet, 1H, CH = CH, J = 15.3 Hz), and 7.937.96 (multiplet, 2H, ArH). 13C NMR spectra (15.6 MHz, DMSO-d6) display peaks at δ16.12, 21.67, 48.12, 126.79, 126.87, 127.13, 128.06, 128.55, 128.60, 129.52, 130.88, 131.88, 132.38, 135.63, 136.58, and 147.42 ppm. The compound's molecular weight was found to be 349.4 m/z (M +H) + using LC-MS (ESI).



Compound (9f)

• (E)-3-(1-benzyl-2-(methylthio)-1H-imidazol 4-yl)-1-(naphthalen2-yl) prop-2-en-1-one (9g)

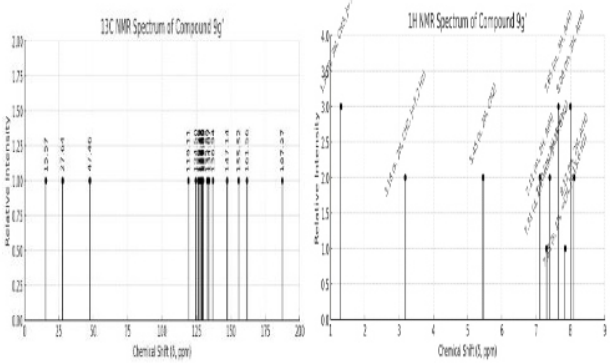
Yield: 60%, Melting point range = 216-218 oC. 1H NMR spectrum was 500 MHz in DMSO d6. Chemical shifts: 2.55 (singlet, 3H, SCH3), 5.58 (singlet, 2H, CH2), 7.197.24 (multiplet, 2H, ArH), 7.34–7.48 (multiplet, 2H, ArH), 8.50 (doublet, 1H, CH = CH, J = 15.6 Hz), 7.667.73 (multiplet, 2H, ArH), 8.53 (multiplet, 5H, ArH), and 9.01 (singlet, 1H, imidazole). The 13C NMR spectrum (126 MHz-DMSO d6) shows chemical shifts (δ) in parts per million (ppm) at the following values: 116.87, 117.05, 119.56, 123.15, 126.02, 129.23, 129.30, 148.39, 148.60, 150.63, 163.07, 163.68, 165.05, 167.77 as observed in Figure. The compound's molecular weight was found to be 385.5 m/z (M +H) + using LC-MS (ESI).



Compound (9g)

• (E)-3-(1-benzyl-2-(ethylthio)-1H-imidazol-4yl)-1-(naphthalen-2- yl) prop-2-en-1-one (9g')

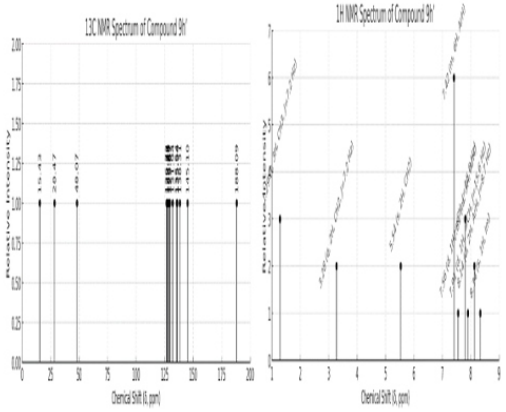
Yield :57%, Melting point (MP) = 103 -105 oC , 1H NMR (DMSO d6 at 300 Mhz): The following are the chemical shifts (δ) expressed in parts per million (ppm): 1.28–1.32 (triplet, 3H, methyl group, J = 7.2 Hz), 3.143.22 (singlet, 2H, methylene group, J = 7.2 Hz), 5.45 (singlet, 2H, methylene group), 7.10–7.12 (multiplet, 2H, aromatic protons), 7.28–7.33 (doublet, 1H, CH = CH, J = 15.6 Hz), 7.37–7.42 (multiplet, 2H, aromatic protons), 7.60–7.70 (multiplet, 3H, aromatic protons), 7.82–7.87 (singlet, 1H, CH = CH, J = 15.6 Hz), 7.94–8.06 (multiplet, 3H, aromatic protons), 8.099–8.12 (multiplet, 2H, aromatic protons). 13C NMR (75 MHz-DMSO d6): The following were the chemical shifts (δ) expressed in parts per million (ppm): The following numbers were 15.57, 27.64, 47.46, 119.115, 124.50, 126.56, 127.42, 128.17, 128.88, 129.05, 129.40, 129.65 as shown in Figure



Compound (9g')

• (E)-1-([1,1′-biphenyl]-4-yl)-3-(1-benzyl-2(ethylthio)-1Himidazol-4-yl) prop-2-en-1-one (9h')

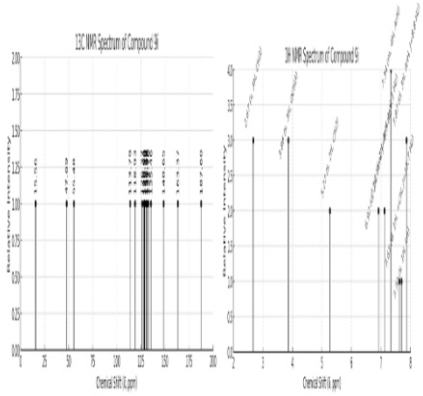
Yield :55%; Melting point = 196–201 ◦C; 1H NMR (200 MHz-DMSO d6): δ (ppm)1.26–1.31 (triplet, 3 hydrogen atoms, methyl group, J = 7.2 Hz), 3.24–3.31 (quartet, 2 hydrogen atoms, methylene group, J = 7.2 Hz), 5.54 (singlet, 2 hydrogen atoms, methylene group), 7.32–7.51 (multiplet, 6 hydrogen atoms, aromatic ring), 7.52–7.60 (doublet, 1 hydrogen atom, CH = CH group, J = 15.6 Hz), 7.732–7.88 (multiplet, 3 hydrogen atoms, aromatic ring), 7.88–7.93 (doublet, 1 hydrogen atom, CH = CH group, J = 15.6 Hz), 8.12–8.152 (doublet, 2 hydrogen atoms, J = 8.7 Hz), 8.262–8.42 (singlet, 1 hydrogen atom, imidazole); 13C NMR(75 MHz-DMSO d6): δ (ppm), 15.43, 28.47, 48.07, 126.72, 127.33, 127.45, 127.512, 128.42, 128.49, 129.37, 129.47, 129.572, 129.619, 131.944, 135.944, 135.54, 138.311, 145.103, 188.09; LC-MS (ESI): 425.3 (M + H)+[37].



Compound (9h')

• (E)-3-(1-benzyl-2-(methylthio)-1H-imidazol 4-yl)-1-(4- methoxyphenyl) prop-2-en-1-one (9i)

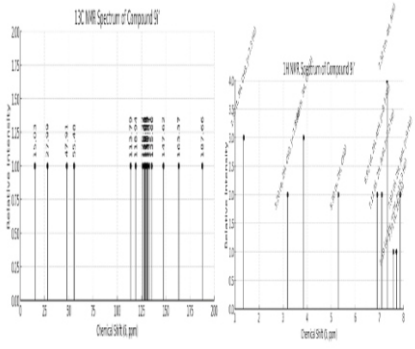
Yield :78%; Melting point = 150–157 ◦C; 1H NMR spectrum (300 MHz-DMSO d6) shows peaks at the following chemical shifts: 2.67 ppm (singlet, 3H, CH3), 3.86 ppm (singlet, 3H, OCH3), 5.27 ppm (singlet, 2H, Ch2), 6.90–6.93 ppm (doublet, 2H, ArH, J = 8.7 Hz), 7.11–7.13 ppm (doublet, 2H, ArH, J = 6.10 Hz), 7.277.40 ppm (multiplet, 4H, ArH), 7.58–7.68 ppm (doublet, 1H, HC = CH, J = 15.7 Hz), 7.70 ppm (singlet, 1H, imidazole), 7.85–7.88 ppm (doublet, 3H, ArH, J = 8.8 Hz). 13C NMR spectrum (75 MHz, CDCl3) shows peaks at the following chemical shifts: 15.56, 47.89, 55.48, 113.79, 118.93, 126.37, 128.08, 128.54, 129.10, 130.59, 130.92, 131.39, 132.70, 135.46, 148.65, 163.37, 187.60 ppm. The LC-MS (ESI) analysis shows: 365.4 (M + H) + and 389.4 (M + Na) +.



Compound (9i)

• (E)-3-(1-benzyl-2-(ethylthio)-1H-imidazol-4 yl)-1-(4- methoxyphenyl) prop-2-en-1-one (9i')

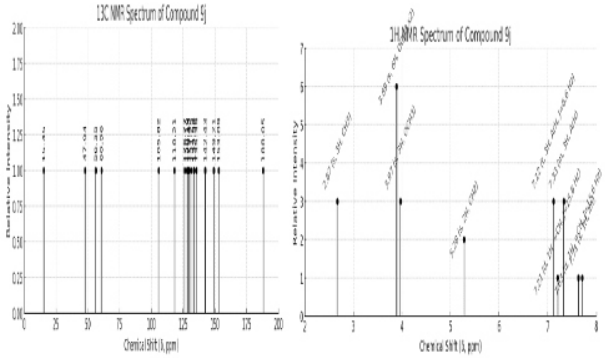
Yield: 78%. Melting point = 143-145 oCelsius. 1H NMR spectrum (measured at 300 MHz using DMSO-d6 as the solvent) shows peaks at chemical shifts (δ) of 1.35-1.39 (triplet, 3 hydrogen atoms, methyl group, coupling constant J = 7.3 Hz), 3.16-3.23 (quartet, 2 hydrogen atoms, methylene group, J = 7.3 Hz), 3.86 (singlet, 3 hydrogen atoms, methoxy group), 5.30 (singlet, 2 hydrogen atoms, methylene group), 6.90-6.93 (doublet, 2 hydrogen atoms, aromatic protons, J = 8.7 Hz), 7.097.12 (doublet, 2 hydrogen atoms, aromatic protons, J = 7.2 Hz), 7.26-7.38 (multiplet, 4 hydrogen atoms, aromatic protons), 7.56-7.61 (doublet, 1 hydrogen atom, vinyl proton, J = 15.3 Hz), 7.71 (singlet, 1 hydrogen atom, imidazole), and 7.85-7.88 (doublet, 2 hydrogen atoms, aromatic protons, J = 9 Hz). The 13C NMR spectrum (measured at 75 MHz using CDCl3 as the solvent) shows peaks at chemical shifts (δ) of 15.03, 27.99, 47.91, 55.48, 113.79, 118.94, 126.32, 128.02, 128.68, 129.07, 130.59, 130.93, 131.22, 132.91, 135.66, 147.62, 163.37, and 187.66. A molecular ion peak of 379.4 (M + H) + in the LC-MS (ESI) analysis.



Compound (9i')

• (E)-3-(1-benzyl-2-(methyl thio)-1Himidazol-4-yl)-1-(3,4,5- trimethoxy phenyl) prop-2en-1-one (9j)

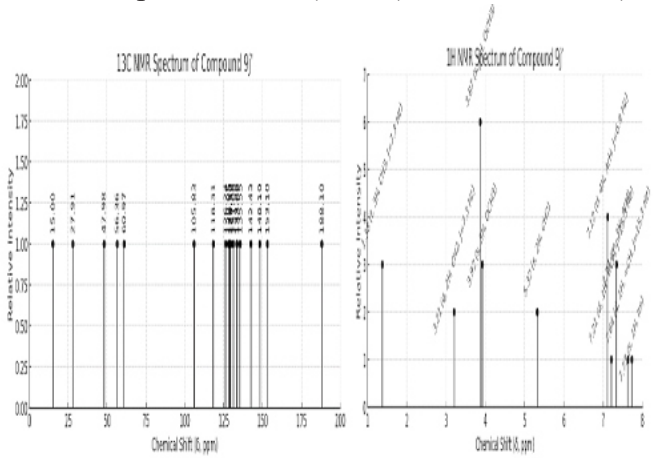
Yield ;86% ,Melting point =171–177 °C. 1H NMR spectrum (400 MHz, DMSO-d6), peaks are seen at the specified chemical shifts: 2.67 ppm (singlet, 3H, CH3), 3.89 ppm (singlet, 6H, OCH3), 3.97 ppm (singlet, 3H, OCH3), 5.28 ppm (singlet, 2H, CH2), 7.11–7.13 ppm (triplet, 3H, ArH, J = 6.6 Hz), 7.18–7.23 ppm (doublet, 1H, HC = CH, J = 15.6 Hz), 7.28–7.38 ppm 3H, ArH), 7.60–7.66 ppm (doublet, 1H, HC = CH, J = 15.6 Hz), and 7.71 ppm (singlet, 1H, imidazole). The 13C NMR spectrum (80 MHz, CDCl3) shows peaks at chemical shifts of 15.45, 47.94, 56.35, 60.96, 105.82, 118.21, 126.27, 128.10, 129.13, 129.43, 131.25, 133.36, 133.57, 135.39, 142.43, 149.21, 153.09, and 188.05 ppm. LC-MS (ESI) analysis m/z 425.4 (M + H) + and 447.4 (M + Na) +.



Compound (9j)

• (E)-3-(1-benzyl-2-(ethylthio)-1H-imidazol-4 yl)-1-(3,4,5- trimethoxy phenyl) prop-2-en-1-one (9j')

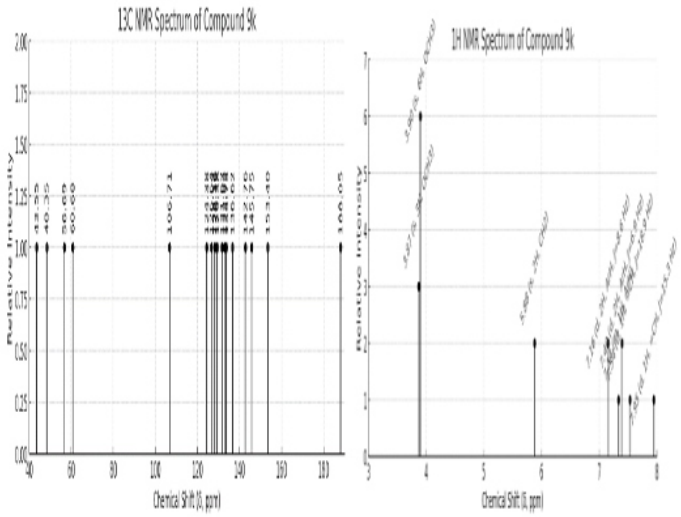
Yield :61%, Melting point range =72-94 ◦C. The 1H NMR spectrum (300 MHz-DMSO d6) shows peaks at δ (ppm) 1.35-1.40 (t, 3H, CH3, J = 7.3 Hz), 3.17-3.24 (q, 2H, CH2, J = 7.3 Hz), 3.87 (s, 6H, OCH3), 3.92 (s, 3H, OCH3), 5.32 (s, 2H, CH2), 7.10-7.13 (d, 4H, ArH, J = 6.9 Hz), 7.18-7.24 (d, 1H, HC = CH, J = 15.3 Hz), 7.29-7.39 (m, 3H, ArH), 7.61-7.66 (d, 1H, HC = CH, J = 15.3 Hz), and 7.73 (s, 1H, imidazole). 13C NMR spectrum (75 MHz, CDCl3) shows peaks at δ (ppm) 15.00, 27.91, 47.98, 56.36, 60.97, 105.83, 118.31, 126.24, 128.05, 129.10, 129.52, 131.08, 133.36, 133.62, 135.55, 142.43, 148.19, 153.10, and 188.10. Molecular ion peak at 439.5 (M + H) + in the LC-MS (ESI) analysis.



Compound (9j')

• (E)-methyl 1-benzyl-5-(3-oxo-3-(3,4,5-tri methoxyphenyl) prop-1-en-1-yl)-1H-imidazole-2 sulfinate (9k)

Yield 87%; Melting point = 169–177 ◦C; 1H NMR spectrum (300 MHz-DMSO d6) shows peaks at the following chemical shifts: 3.87 ppm (singlet, 3H, OCH3), 3.90 ppm (singlet, 6H, OCH3), 5.88 ppm (singlet, 2H, Ch2), 7.11–7.20 ppm (doublet, 2H, ArH, J = 8.6 Hz), 7.30–7.37 ppm (multiplet, 1H, ArH), 7.387.41 ppm (doublet, 2H, ArH, J = 6.9 Hz), 7.38–7.43 ppm (multiplet, 2H, ArH), 7.51–7.56 ppm (doublet, 1H, HC = CH, J = 18.9 Hz), and 7.92–7.97 ppm (doublet, 1H, HC = CH, J = 15.3 Hz). 13C NMR spectrum (75 MHz-DMSO d6) shows peaks at the following chemical shifts: 43.55 ppm, 48.35 ppm, 56.69 ppm, 60.68 ppm, 106.71 ppm, 124.38 ppm, 126.64 ppm, 128.16 ppm, 128.35 ppm, 129.38 ppm, 131.62 ppm, 132.83 ppm, 133.75 ppm, 136.62 ppm, 142.76 ppm, 145.75 ppm, and 153.40 ppm. The LC-MS (ESI) analysis shows m/z 457.5 (M + H) + and m/z 479.4 (M + Na) +.



Compound (9k)

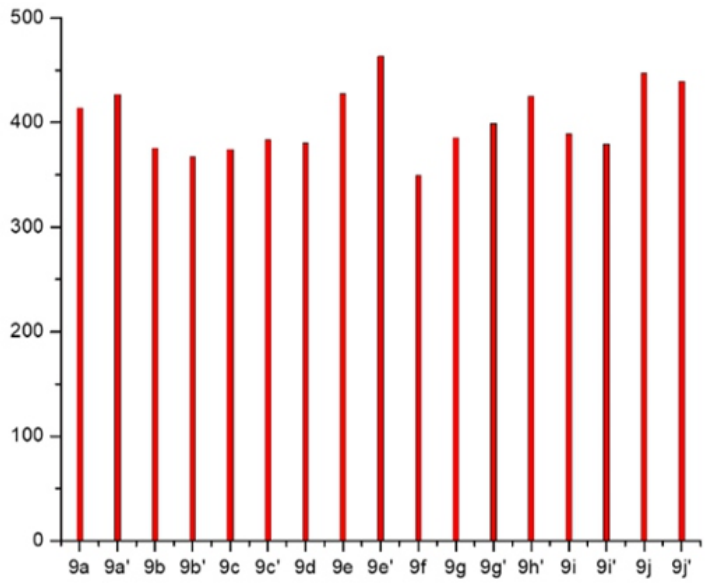


Figure 2: Mass Spectrometry of Synthesized compounds

3.2 Biological Evaluation

The compounds (9a–9j') tested against a range microorganisms for their antifungal and antibacterial qualities. The MTT colorimetric assay yielded the MICs, which at a rate of 66.11% provided insightful information about the compounds' potential as antibacterial agents.

• Antibacterial Assessment

The antibacterial evaluation involved prominent bacterial strains, including S. aureus, B. cereus, E. coli, and P. aeruginosa. The components were examined at a 50 µg/mL concentration in DMSO, and their inhibitory effects were assessed in Mueller-Hinton (MH) medium. Visual analysis was used to establish the minimum concentration (MIC) at which microbial growth was suppressed [38,39]. The results were summarized in Table 3 below:

Table 3: Antibacterial activity of synthesized compounds (9a-9j')

Compounds	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*
9a	250	200	200	250
9b	250	125	62.5	250
9c	250	250	200	250
9d	250	100	100	125
9e	125	50	50	125
9f	125	50	62	100
9g	100	70	56	200
9i	200	50	250	200
9j	100	200	100	100
9k	250	150	62.5	250
9a'	200	160	100	50
9b'	50	100	50	50
9c'	10	60	10	160
9e'	50	100	25	140
9g'	135	200	100	250
9h'	150	250	200	210
9i'	200	170	100	230
9j'	140	180	250	200

The synthetic compounds, 9a–9j, demonstrated antibacterial activity against a range of bacterial strains, including S. aureus, B. cereus, E. coli, and P. aeruginosa. The Minimum Inhibitory Concentrations (MIC), expressed in micrograms per millilitre (µg/mL), were established for each compound against each species of bacteria.

Among the compounds, 9b exhibited noteworthy antibacterial efficacy, particularly standing out against B. cereus and E. coli, with MIC values of 125 µg/mL and 62.5 µg/mL, respectively as shown in Figure 5. Similarly, compound 9e demonstrated significant activity against all bacterial strains tested, with MIC values ranging from 50 µg/mL to 125 µg/mL. Compound 9c consistently displayed inhibitory effects against S. aureus, B. cereus, and P. aeruginosa, with MIC values of 250 µg/mL across all strains as shown in the figure below. Compounds 9g and 9j' showcased varied activity against different bacterial strains, hinting at potential selectivity. Notably, 9g exhibited a lower MIC against E. coli (56 µg/mL) compared to other strains, while 9j' demonstrated higher potency against P. aeruginosa with an MIC of 250 µg/mL.

Compound 9c' demonstrated low MIC values across all bacterial strains, indicating broad-spectrum antibacterial activity. Conversely, 9h' exhibited high MIC values, suggesting lower efficacy against the tested bacterial strains as represented in Figure 6. Overall, the synthesised compounds exhibited varied antibacterial properties, with some demonstrating potential effectiveness against certain bacterial strains. The findings provide useful insights into the possible uses of these compounds as antibacterial agents, indicating the necessity for additional research and advancement.

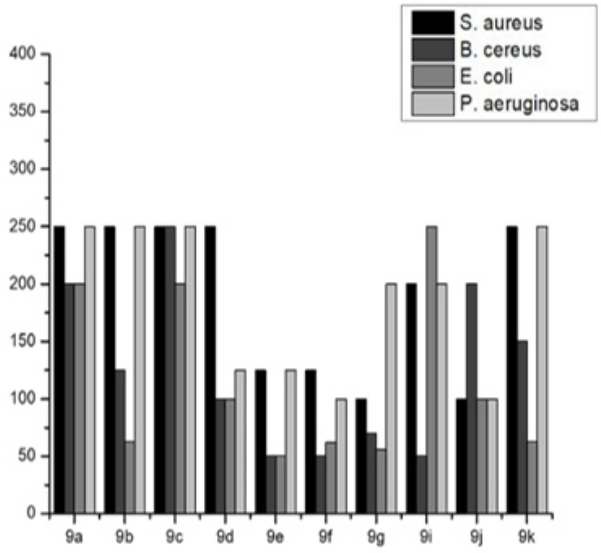


Figure 3: Antibacterial Assessment for compounds (9a-9k)

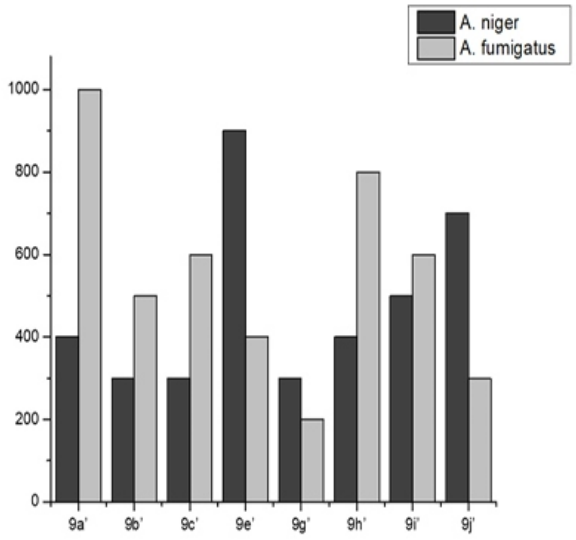


Figure 6: Antifungal Assessment for compounds (9a'-9j')

4. Discussion

4.1 Synthesis of Imidazole-based chalcone derivatives

The synthesis of imidazole-based chalcone derivatives involved a multi-step approach, resulting in well-defined compounds. Characterization using Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry validated their chemical structures, aligning with similar methodologies employed in previous studies [40-45].

Comparing these findings with existing research, the methodology employed shared similarities with other studies exploring chalcone derivatives. Tran et al., (2012), Modzelewska et al., (2006), Baviskar et al., (2009), and Vogel et al., (2010) used a multi-step synthesis method that included Claisen-Schmidt condensation. They then analysed the chalcone derivatives using NMR and mass spectrometry for investigation. This convergence in synthetic strategies reinforces the reliability and reproducibility of the presented work [46-49]. Furthermore, Jaisiewicz et al., (2023); and Dorababu et al., (2020) utilized new bioactive compounds, a methodology based on combining two molecules with biological properties into a new hybrid molecule used to design and synthesize a series of ten indole derivatives bearing imidazole, benzothiazole-2-thione, or benzoxazole-2-thione moieties at the C-3 position [50,51]. The oxidation step using Oxone to obtain the oxidized methyl sulfonyl molecule 9k adds a distinctive element to the synthesis, contributing to the structural diversity of the final compounds. This methodology aligns with Liu et al., (2013) work on incorporating oxidation steps to enhance the chemical diversity of chalcone derivatives, indicating a shared focus on structural intricacies [52].

The verification of the synthesized compounds' structural properties through nuclear magnetic resonance (1H NMR, 13C NMR) and mass spectrometry studies was a crucial aspect, ensuring the reliability of the results. Similar verification protocols are echoed in studies by Oskuei et al., (2021) [53] and Sangeetha et al., (2021), underlining the importance of robust characterization techniques for novel compounds [54].

Overall, the synthesis process showcased a detailed and well-supported methodology, incorporating innovative elements like oxidation stages and thorough structural verification, paving the way for potential applications in medicinal chemistry.

4.2 Antimicrobial Study

The antimicrobial evaluation of the synthesized compounds against bacterial and fungal strains provided valuable insights into their potential applications. The selective antibacterial efficacy of compound 9b against B. cereus and E. coli, as well as the broad-spectrum antibacterial activity of compound 9c, aligns with similar observations in studies by Osmaniye et al., (2018) and Tratrat et al., (2019) [55,56]. These consistent findings contribute to establishing a robust foundation for the antibacterial potential of imidazole-based chalcone derivatives.

Moreover, the antifungal assessment reveals promising results, with compounds 9b and 9e demonstrating notable efficacy against A. niger and A. fumigatus. This aligns with the work of Tank et al., (2022); Abonia et al., (2012); Pankaj et al., (2020), who also reported significant antifungal activity in chalcone derivatives against A. niger [57-59] Additionally, the selectivity observed in compound 9j' mirrors findings in Sattu et al., (2018), suggesting a nuanced impact on different fungal strains [60].

The reliability of antimicrobial outcomes, in comparison to comparable studies, underscores the potential of these compounds in combating bacterial and fungal diseases, laying the groundwork for the development of innovative antibacterial medications.

Conclusion

In conclusion, the synthesis and characterization of imidazole-based chalcone derivatives, coupled with their comprehensive antimicrobial evaluation, offer a promising avenue for the development of potential therapeutic agents. The methodological alignment with comparable studies and the consistent observations in antibacterial and antifungal activities contribute to the robustness and reliability of the presented research. The structural diversity achieved through oxidation steps adds an innovative dimension to the synthesis. These findings underscore the potential of imidazole-based chalcone derivatives as effective agents against bacterial and fungal infections. Future research endeavours can leverage these insights to further optimize and advance the development of novel antimicrobial drugs.

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