International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : http://ijpsdronline.com/index.php/journal

Research Article

Investigating Bioactive Compound Extraction and Evaluation of *Xantolis tomentosa* based on Response Surface Methodology

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ARTICLE INFO

Article history:

Received: 10 February, 2026

Revised: 03 March, 2026

Accepted: 10 March, 2026

Published: 30 March, 2026

Keywords:

Xantolis tomentosa, Response Surface Methodology, Phytochemicals, Antioxidant activity, biomedical property.

DOI:

10.25004/IJPSDR.2026.180201

ABSTRACT

Xantolis tomentosa (Roxb.) Raf. is an ethnomedicinal plant traditionally used for treating inflammatory and infectious disorders. In the current research, Response Surface Methodology (RSM) was used to optimize the extraction of bioactive compounds from *X. tomentosa* leaves. A Box–Behnken Design (BBD) was applied to evaluate the effects of solvent concentration (40–80%), extraction temperature (40–70°C), and extraction time (20–60 min) on phytochemical yield and biological activity. The optimized extraction conditions yielded elevated levels of total flavonoids (11.8 mg/g), total phenolics (0.98 mg/g), and total terpenoids (0.48 mg/g). The extract demonstrated strong antioxidant activity, with DPPH radical scavenging inhibition ≥ 83.2%, along with significant antimicrobial activity (maximum inhibition zone: 20.14 mm). GC–MS analysis identified multiple compounds with potential biological activity, supporting the observed antioxidant and antimicrobial properties. These findings demonstrate that statistically optimized extraction significantly enhances the bioactivity of *X. tomentosa* and provide scientific validation for its traditional medicinal applications.

Introduction

Phytoconstituents, which contribute to the color, fragrance, and flavor of plants, are naturally occurring, plant-based compounds recognized for their therapeutic potential.[1] Phytoconstituents are metabolic compounds that are not non-essential nutrients.[2] Phytoconstituents frequently display substantial preventative and protective effects against diseases.[3] This includes antioxidant, antiviral, antibacterial, and antifungal activities, highlighting their value.[4] Given the diversity of their pharmacological activities, phytoconstituents have been popular candidates for developing new drugs and functional foods. The global herbal product market has expanded vastly over the last few decades, indicating increasing interest in bioactive compounds from nature for health and industry use.[5] Many plant compounds exhibit both antimicrobial and antioxidant activity, both of which are important properties in the pharmaceutical and food preservation industries.[6] Antioxidants, in particular, act by scavenging reactive oxygen species, reducing oxidative stress, and protection against lipid peroxidation and cellular damage.[7]

Xantolis tomentosa (Roxb.) Raf., a member of the family Sapotaceae, comprises more than 53 genera and approximately 1100 species.[8] These are traditionally used to treat inflammation, pain, fever, respiratory discomfort, diarrhea, and as diuretics, expectorants, and ophthalmic agents.[9] Plants belonging to the Sapotaceae family are recognized as rich sources of phenolic and flavonoid compounds with strong antioxidant activity.[10] Specifically, *X. tomentosa* has been used in folk medicine to manage osteoarthritis and rheumatoid arthritis. [11] Despite its wide traditional use, systematic optimization of its bioactive compound extraction and evaluation of its

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Table 1: Comparison of existing literature from the Sapotacea family

Author	*Objective*	*Extract used*	*Technique*	*Outcomes*	***Limitation***
Divyalakshmi and Thoppil, [12]	To synthesize and characterize silver nanoparticles using yellow mangosteen leaf extract and evaluate their biological activity.	*Yellow mangosteen leaf extract*	Green synthesis of AgNPs; UV–Vis, FTIR, SEM, and XRD characterization; antioxidant & antimicrobial assays.	Successfully synthesized stable AgNPs with significant antioxidant and antimicrobial properties.	Study limited to in-vitro analysis; no in-vivo toxicity or biocompatibility tests performed.
Bachhar, V. *et al*. [13]	To identify bioactive phytoconstituents, nutritional components, and antioxidant activity of *Calyptocarpus vialis*.	*Calyptocarpus vialis* whole plant extract	GC–MS, proximate analysis, DPPH and FRAP assays.	Presence of phenolic compounds, vitamins, minerals, and high antioxidant activity confirmed.	Study lacks pharmacological or in-vivo correlation for bioactivity validation.
Choudhary, N. *et al*. [14]	To compare conventional and microwave-assisted extraction methods for *Chenopodium album* phytoconstituents.	*Chenopodium album* leaves extract	Conventional solvent extraction vs. Microwave-assisted extraction (MAE); phytochemical analysis.	MAE yielded higher extraction efficiency and more active compounds than conventional methods.	Focused only on extraction efficiency; biological activity not tested.
Neethu, S. *et al*. [15]	To assess phytoconstituents and develop standardization protocol for 'Nayopayam Kwatha'.	*Polyherbal Ayurvedic formulation*	Phytochemical screening, TLC fingerprinting, physicochemical analysis.	Identified major phytoconstituents and established standard parameters for quality control.	Did not evaluate pharmacological efficacy or clinical validation.
Dunachie, S.J. *et al*.[16]	To discuss challenges in estimating the global burden of antimicrobial-resistant (AMR) infections.	–	-	Highlighted data gaps, variability in surveillance, and regional differences in AMR reporting.	Lack of standardized global surveillance systems limits accuracy of AMR burden estimates.
Carrique-Mas, J.J. *et al*.[17]	To estimate antimicrobial usage in humans and animals in Vietnam.	–	Data collection and statistical modeling from national databases.	Quantified total antimicrobial consumption; revealed high usage in livestock.	Limited by incomplete data reporting and regional underrepresentation.
Wali, A.F. *et al*.[18]	To identify edible medicinal plants and assess their socio-economic contributions	Various edible medicinal plants	-	Provided ethnomedicinal, nutritional, and economic importance of edible plants.	Lacks experimental validation and chemical analysis of bioactives.
Alemu, M. *et al*. [19]	To conduct ethnobotanical study of medicinal plants in Habru District, Ethiopia.	Traditional medicinal plants (various species)	Field interviews, quantitative ethnobotanical indices (UV, ICF).	Identified 64 medicinal species with high local importance; documented indigenous knowledge.	Ethnobotanical data only; no phytochemical or pharmacological verification.
Xiang, Y. *et al*.[20]	To analyze secondary metabolites and antioxidant activity of different parts of *Alpiniae oxyphylla Miq.*	*Alpiniae oxyphylla* fruits, leaves, roots	LC–MS, HPLC, DPPH and ABTS assays.	High phenolic and flavonoid contents; fruit extract showed strongest antioxidant activity.	Study confined to in-vitro antioxidant activity; mechanism not elucidated.
Sameeksha, S. *et al*.[21]	To explore the therapeutic and culinary potential of *Madhuca longifolia* flowers and identify research gaps.	*Madhuca longifolia* flower extract	-	Highlighted bioactive compounds with potential antioxidant, antimicrobial, and nutritional value.	Lacks experimental and clinical validation; based mainly on secondary data.

antioxidant and antimicrobial properties remains limited. According to existing reports, this is the first systematic study designed to refine the extraction process for bioactive constituents from *X. tomentosa* using RSM. Previous studies have focused mainly on the qualitative or preliminary screening of the plant's phytochemicals, without statistically optimizing the process parameters that influence extraction efficiency. This research not only determines the important variables that impact the yield of phenolics and flavonoids but also relates the extraction condition to biological activity (antioxidant and antimicrobial) using a predictive RSM. The optimized extract demonstrates increased bioactivity, indicating that *X. tomentosa* has potential as a newly discovered natural source of pharmacologically active compounds for therapeutic and nutraceuticals applications.

Table 1 summarizes recent advancements in phytochemicals research and bioactivity assessment of medicinal plants, with a focus on their antioxidant, therapeutic, and antimicrobial capabilities. Advanced analyses using GC/MS, HPLC, and various techniques like spectroscopy have generally reported positive results for the characterization of bioactive compounds. However, most of the studies reviewed have a common limitation - dependence on laboratory assays, low variability in extraction parameters, and no in vivo or mechanistic rationale and therefore there remains a critical need to establish links between the biological activity of phytochemicals and their phytochemical make-up, as well as optimizing extraction procedures to maximize bioactivity.

The Sapotaceae family has great ethnomedicinal significance, but only a small number of studies have been carried out that have comprehensively assessed its phytochemical capabilities and biological activities. Studies of some species, for example, *Pouteria caimito*,[22] *Madhuca longifolia*,[23] *Alpiniae oxyphylla*,[24] and *Calyptocarpus vialis* [25] indicate their potential as strong antioxidants, antimicrobial agents and potential for therapy due to the presence of many bioactive components like phenolics, flavonoids, terpenoids and hydrocarbons with long-chain carbon backbones. All of the previously mentioned studies have focused primarily on qualitative analysis or relied upon traditional methods of extraction with little to no focus on the factors (solvent systems, temperature, extraction time, etc.) that influence the nature and quantity of phytochemicals obtained.[26] In addition, very few studies have focused specifically on *X. tomentosa*, and to date, there are no comprehensive reports relating extraction parameters to quantitative phytochemical yield or to subsequent antioxidant or antimicrobial efficacy. Although preliminary studies have indicated the presence of secondary metabolites from *X. tomentosa*, no study has utilized RSM or other statistically driven models to optimise the extraction process.[27] This gap in the literature underscores the need for a systematic, optimization-based study that scientifically validates the traditional uses of *X. tomentosa* and maximizes its pharmacological potential through controlled extraction processes.

Although there is abundant historical use of *X. tomentosa* in ethnomedicinal practices for treating inflammatory and infectious diseases, evidence from systematic scientific methods for validating and optimizing its bioactive compounds is sorely lacking. Previous investigations have often focused on preliminary phytochemical screening without considering the important extraction parameters that affect the yield and activity of phytoconstituents, and without linking the efficiency of extraction to the antioxidant and antibacterial potential of phytoconstituents. This knowledge gap limits the scientific understanding and future pharmaceutical applications of *X. tomentosa* extracts. Thus, there is a clear need to validate and optimize the extraction processes for the major Phytoconstituents and to evaluate the biological activity of the resultant extracts, offering evidence-based justification for their medicinal use.

In the present pharmaceutical landscape, the demand for safe, effective and naturally derived therapeutic agents is rapidly increasing due to the growing challenges of antimicrobial resistance, adverse effects of synthetic drugs, and the need for potent natural antioxidants. Numerous plant species containing high concentrations of phenolic compounds, flavonoids and terpenoids, which make them attractive candidates for anti-inflammatory and antimicrobial medications as well as being potential free-radical scavengers, have become increasingly popular as natural sources of these metabolites for use in the formulation of pharmaceutical products. By selecting these compounds from *X. tomentosa*, it is possible to develop a framework that allows for consistent pharmacological activity through a validated set of guidelines for the successful extraction of the desired constituents. As such, creating and validating extraction conditions and validating the antioxidant and antimicrobial properties of the plant will yield significant pharmaceutical benefits and provide a basis for innovative applications, including herbal formulations, nutraceuticals, and adjunctive therapies.

X. tomentosa was characterised and evaluated for a few of its bioactive properties through chemical analysis (Phytochemical and GC- MS), antioxidant activity as well as antimicrobial properties, and was confirmed through RSM method validation.

Materials And Methods

The overall methodological framework Fig. 1 involves a systematic approach combining experimental extraction, phytochemical analysis and statistical optimization to evaluate as well as enhance the bioactive potential of *X.*

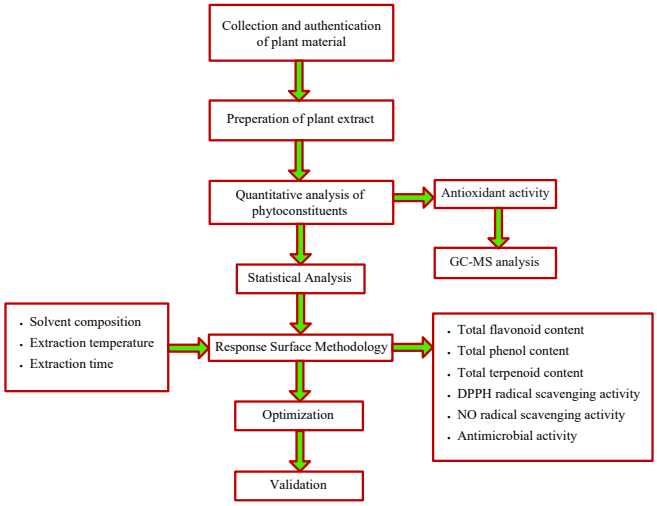


Fig. 1: Proposed methodology

tomentosa leaf extracts. Freshly collected and verified plant material was shade-dried, followed by solvent extractions in a Soxhlet extractor, each with a greater polarity solvent. Crude extracts were then evaluated for concentrations of relevant phytoconstituents, including flavonoids, phenols, and terpenoids. The extracts were further assessed for biopharmaceutical activities through antioxidant and antimicrobial assays before characterizing the predominant bioactive compound(s) using GC–MS. Statistical analyses of experimental data were performed using RSM to optimize the primary parameters. The optimized conditions were experimentally confirmed to further verify model predictions and yield phytochemicals concurrently with potential bioactivity. This integrated analytical approach allows for a scientific evaluation of the phytochemical and therapeutic wealth of extracts of *X. tomentosa*.

Collection and Authentication of Plant Material

Fresh leaves of *X. tomentosa (Roxb.) Raf.*, a medicinal species of the family Sapotaceae, was collected from the natural forest areas located at the outskirts of Idukki District, Kerala, India, in their flowering season (January–March). The plant was identified and authenticated by a trained taxonomist in the Department of Botany, Nesamony Memorial Christian College, Marthandam, Tamil Nadu based on its morphological characteristics. The collected fresh plant material was checked for freshness, as well as the absence of fungal infection or physical damage. Only healthy and disease-free leaves were used for experimentation.

Preparation of Plant Extracts

The collected leaves were thoroughly rinsed under running tap water and subsequently washed with distilled water to remove dust, soil, and other surface impurities. The leaves were dried in the shade at standard room conditions (28 ± 2°C) for about 15-20 days, or until constant weight was reached, so that thermolabile phytoconstituents were preserved. After drying, the leaves were ground to obtain a coarse powder, which was stored in airtight containers maintained under dry conditions. Approximately 100 g of each powdered sample was sequentially extracted using a Soxhlet apparatus in order of polarity; solvents included chloroform, methanol, distilled water, acetone and petroleum ether. For each extraction, the extraction continued until the solvent was colorless, at which point solvation extraction of phytochemicals was complete. The solvent of each sample was removed after filtering through Whatman No. 1 paper by rotary evaporator under reduced pressure to obtain concentrated crude extracts. The extracts were placed in sterile vials and maintained at 4°C prior to analysis.

These extracts were subsequently utilized for preliminary phytochemical screening, quantitative estimation of key bioactive compounds, antioxidant and antimicrobial activity assays, and GC–MS analysis for compound identification.

Phytochemical screening

The preparations were evaluated for the presence of phytochemical uisng the methods specified by Harborne (1973).[28] The phytochemical components tested include: steroids; reduced sugar; alkaloids; phenolic compounds; flavonoids; phytosterols; quinones; xanthoprotein; carboxylic acids; coumarin glycosides; and terpenoids.

Quantitative determination of phytoconstituents

Determination of Flavonoids

Around 10 g of the dried sample was subjected to repeated extraction using 100 mL of 80% methanol at ambient temperature. The extracts were then filtered and placed in a pre-weighed crucible. The filtrate was evaporated to dryness until a constant mass was reached. The crystalline flavonoids crystallized in the crucible and were carefully collected. Mass of residue was noted, and the flavonoid content of the leaf sample was obtained based on equation (1).[29]

$$\%\,Flavanoids = \frac{Weight\ of\ Flavanoids}{Weight\ of\ Sample} \times 100 \quad (1)$$

Determination of terpenoids

Ten grams of crude sample underwent ethanol extraction (9 mL) for 24 hours, followed by extraction with 10 mL of petroleum ether in a separating funnel. The ether extract was isolated, dried, and its mass recorded before and after drying. The total terpenoid content was obtained from equation (2).[30]

$$\%\,Terpenoids = \frac{Weight\ of\ Terpenoids\ initial - Final}{Weight\ of\ Sample} \times 100 \quad (2)$$

Determination of Phenols

About 10 g of the crude sample taken was mixed with ether, followed by the addition of 5% sodium hydroxide and 1:1 hydrochloric acid. Phenols were precipitated, which were then dried and weighed. From the observation value, the total phenol content was determined by the following equation (3).[31]

$$\%\ Phenols = \frac{Weight\ of\ Phenols}{Weight\ of\ Sample} \times 100 \quad (3)$$

Antimicrobial Activity

The antimicrobial potential of the plant extracts was determined by the disc diffusion technique as per the Kirby-Bauer method.[32] A lawn culture was developed by spreading the microbial suspension over the surface of solidified agar in sterile Petri plates. Sterile paper discs were impregnated with 10 µL of the essential oil and gently placed on Muller-Hinton agar plates. Standard antibiotic discs were also placed on the same plates as positive controls. The plates were incubated for 24 hours at 37°C, and the inhibition zone diameters were measured (in mm) after incubation.

Antioxidant Activity

To characterize the antioxidant potential, Nitric oxide NO and DPPH-based free radical scavenging assay were used on the leaf extracts of *X. tomentosa (R)*. The leaf extracts were diluted to made different concentration samples such as 500, 250, 100, 50, and 10 µg/mL then the 0.3ml of the sample was added to 0.1 mM of DPPH in methanol. The samples were then shaken thoroughly and allowed to sit for 30 minutes at normal laboratory temperature. Then, using ascorbic acid as the reference, the absorbance at 517nm was measured with a UV-VIS spectrophotometer. Likewise, the scavenging assay with NO was performed at different concentrations of samples (500, 250, 100, 50 and 10 µg/mL) prepared from 50 mg/mL of the leaf extracts diluted with dimethyl sulphoxide (DMSO). The samples were then mixed with an equal quantity of freshly prepared Griess reagent (150µl). Following the reaction step, 100 µL of the mixture was placed into a 96-well microplate and the absorbance was measured at 540 nm with a microplate after 540 nm after 30 minutes The activity of the samples was recorded using equation (4).[33]

$$Radial\ scavenging\ activity\ \% = \{(Absorbance_{control} - Absorbance_{sample}) / Absorbance_{control}\} \quad (4)$$

GC-MS Analysis

The GC/MS system was used to analyze the extracts that were separated using column chromatography on a Shimadzu QP-2010 Plus GC/MS system equipped with a TD-20 thermal desorption system. The column temperature was programmed from an initial temperature of 100°C at a rate of 15°C/min to a final temperature of 280°C, and this final temperature was held for 40 minutes with an interface temperature of 270°C and an ion-source temperature of 230°C. The analytes were detected using a mass range of 20 to 520 amu; this was followed by identification based on comparison of the experimental spectra with the NIST library.[34]

Optimization of Extraction Conditions Using RSM

The parameters involved in the extraction process influence the number of bioactive compounds extracted, and these parameters were optimized using RSM with a BBD. [35] In this study, three independent variables: solvent concentration, extraction temperature, and extraction time, were identified. The response variables were Total Flavonoid Content (TFC), antioxidant activity, and Total Phenolic Content (TPC).

A total of 30 experimental runs were generated using Design-Expert software, which evaluated all possible combinations of the independent variables. The experimental results were interpreted using a second-order polynomial regression model, which is mentioned in equation (5), to report the correlation between the process variables and the response.

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{1,2} AB + \alpha_{1,3} AC + \alpha_{2,3} BC + \alpha_{1,1} A^2 + \alpha_{2,2} 2B^2 + \alpha_{3,3} C^2 \quad (5)$$

Where, Y denotes the predicted response, A, B, C are coded variables of the independent factors, and α terms represent regression coefficients.

Model adequacy was assessed by ANOVA, and individual term significance by p values ($p < 0.05$). The coefficient of determination (R^2), adjusted R^2, and lack-of-fit tests were used to evaluate the precision of the model fit. The work flow of RSM in this study is shown in Fig. 2.

The results showed that all three factors significantly affected the extraction yield, with solvent concentration and temperature showing the most potent effects. The optimized conditions for maximum phenolic and flavonoid recovery were 75% methanol concentration, 50°C extraction temperature and 40 minutes extraction time. Under the optimized settings, the measured responses closely matched the model predictions, confirming the reliability of the RSM model.

Results And Discussion

Phytochemical Analysis

Phytochemical screening identified the presence of various bioactive compounds, including steroids, reduced sugars, alkaloids, phenols, flavonoids, phytosterols, quinones, xanthoprotein, carboxylic acids, coumarin glycosides, and terpenoids. The extracts obtained with the solvents showed the most incredible diversity of phytochemicals, with the maximum present in the acetone extract and

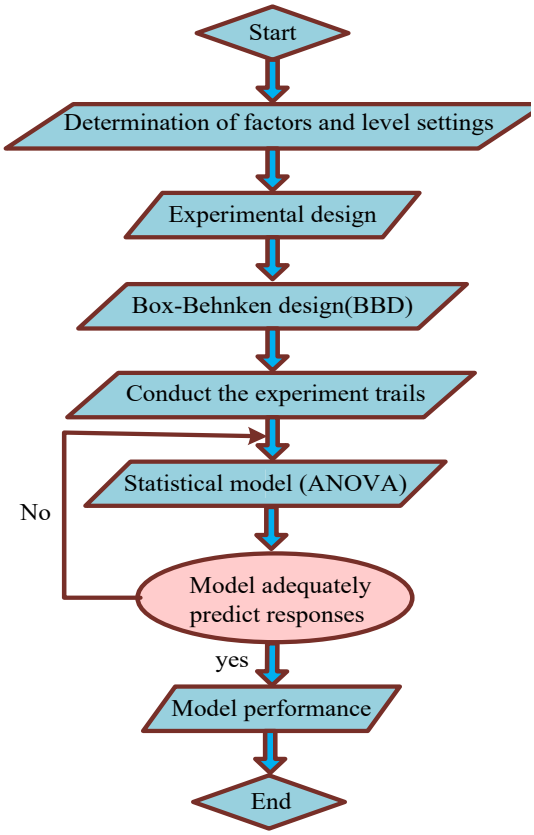


Fig. 2: Flowchart of RSM

the least in the chloroform extract. The outcomes of the qualitative screening are shown in Table 2. The "+" indicates the presence of phytochemical components of interest in the extract, and "–" is used to indicate the absence of phytochemical components in the extract.

The variation in phytochemical constituents among the different solvent extracts is primarily due to the differential polarity of the solvents used. Both acetone and methanol, which are polar solvents, will be able to dissolve oxygen-containing compounds of phytochemicals found in many plants. As a result, there is an increased ability for the plant cell wall to allow for the movement of these materials through its cellular structure, as well as an increased solubility of the phenolic compounds and flavonoids and terpenoids.[36] These factors have contributed to the high diversity of phytochemicals seen in plant materials extracted with acetone and methanol, compared to extracts made with chloroform and petroleum ether. Other studies involving similar plant families have similar solvent-based isolation results, including greater extraction yields of phytochemical groups and bioactive constituents using polar solvents. [36]

Quantitative Analysis of Phytoconstituents

The quantitative analysis of phytochemicals revealed notable variations in the concentration of bioactive compounds in the leaf extract of *X. tomentosa* (R), as shown in Fig. 3. The TFC had a total of 83% indicating a high level of antioxidant compounds that help inhibit free radicals. The Total Terpenoid Content (TTC) was 2.1% corresponding to the compounds that contribute to the anti-inflammatory activity and antimicrobial activity of the plant. The TPC was also 6.2 %, indicating a potent antioxidant and medicinal activity of the extract.

Antioxidant Studies

Antioxidants are essential because they protect the body from harmful molecules such as ROS, RNS, and RCS that can cause cellular damage. Different concentrations of the extracts and standard ascorbic acid are used in this study. Fig. 4 represents antioxidant studies of the extracts demonstrating DPPH and NO scavenging activities in panels (a) and (b), respectively. Maximum percentage of inhibition is noted at 500 µg/mL, and minimum percentage of inhibition is observed at 10 µg/mL. Petroleum ether and aqueous extracts exhibit significant antioxidant activity compared with the others.

Table 2: Phytochemical analysis of the plant extracts

S. No	*Phytochemicals*	*Petroleum ether extract*	*Chloro form extract*	*Acetone extract*	*Metha nol extract*	*Aque ous extract*
1	Steroids	+	-	+	+	+
2	Reduced sugars	+	+	+	+	+
3	Alkaloids	+	+	+	+	+
4	Phenols	-	-	+	+	+
5	Flavanoids	-	-	+	+	+
6	Phytosterols	+	-	+	+	+
7	Quinones	-	-	+	+	+
8	Xanthoprotein	+	+	+	+	+
9	Saponins	-	-	-	-	-
10	Carboxylic acids	+	+	+	-	-
11	Coumarin glycosides	+	+	-	-	-
12	Terpenoids	+	-	+	+	+

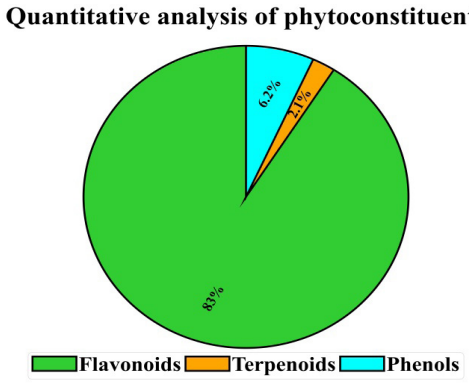


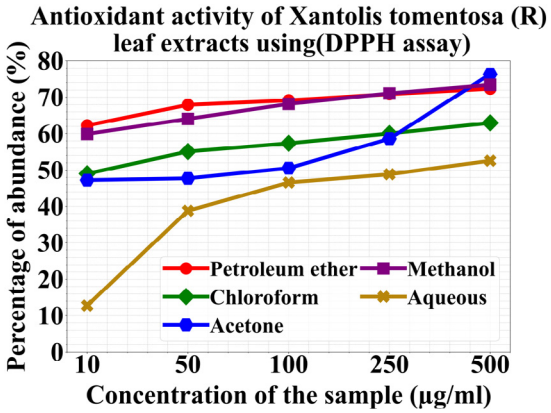
Fig. 3: Quantitative assessment of phytoconstituents

The IC_{50} values indicate the antioxidant potency of different *X. tomentosa* leaf extracts. Lower IC_{50} values correspond to more potent antioxidant activity. From Table 3, the aqueous extract indicated the peak antioxidant activity in the DPPH assay (IC_{50} = 39.32 µg/mL), whereas the petroleum ether extract showed the most vigorous activity in the NO scavenging assay (IC_{50} = 77.3 µg/mL). The antioxidant activity was much lower in acetone and chloroform extracts, as evidenced by their highest IC_0 values. Results demonstrate that the extraction of bioactive compounds in scavenging free radicals is greatly influenced by the extraction solvent.

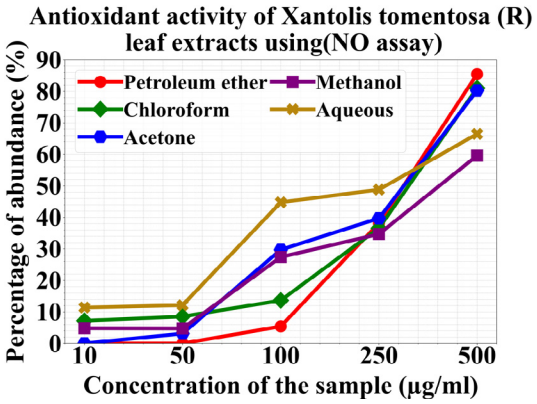
The strong antioxidant properties of the two extract types (Aqueous and Petroleum Ether) are supported by the phytochemical composition of both extracts (which contain a high concentration of flavonoid and phenolic compounds). The antioxidant effects of these compounds result from three mechanisms (hydrogen donation, radical scavenging, and metal chelation). Therefore, extracts with lower IC_{50} values are more effective at quenching free radicals than extracts with higher IC_{50} values. The strong connection between antioxidant capacity and total phenolic/flavonoid content has been shown in many previous investigations of medicinal plants.[38] The role of solvent polarity in the extraction of antioxidant-rich compounds explains why aqueous extract is superior in its DPPH assay and petroleum extract is superior in its activity as a NO scavenger.[39]

Table 3: IC_{50} values of *X. tomentosa* leaf extracts for DPPH and NO scavenging assays.

Extracts	*IC_{50}*	
	DPPH Assay	*NO Assay*
Petroleum ether extract	81.32	77.3
Chloroform extract	70.66	264.8
Acetone extract	266.7	145.2
Methanol extract	80.51	165.6
Aqueous extract	39.32	112.7



(a)



(b)

Fig. 4: Antioxidant studies, (a) DPPH and (b) NO

Antimicrobial Studies

The antimicrobial studies further supported the bioefficacy of the extracts.

The antibacterial activity and antifungal activity of *X. tomentosa* (R) leaf extracts against selected pathogenic microorganisms are shown in Figs 5 & 6. The extracts were tested against gram-positive bacteria (*Enterococcus spp., Bacillus subtilis*, and *Staphylococcus albus*), gram-negative bacteria (*Enterobacter spp, Pseudomonas aeruginosa,* and. *Klebsiella spp*), and fungal strains (*Aspergillus niger, Candida albicans, and Candida tropicalis).* Among the five extracts tested, the methanol extract exhibited the highest antibacterial activity, producing inhibition zones of approximately 19, 15, and 19 mm against the gram-positive bacteria. For the gram-negative bacteria, both methanol and acetone extracts demonstrated strong inhibitory effects, with inhibition zones ranging between 13 to 19 mm. The antibacterial assay results indicated that acetone and aqueous extracts were much more effective against fungi than the other extracts; the inhibited growth of all fungi tested ranged from 16 to 18 mm. The findings of this study suggest that polar phytoconstituents, particularly phenols and flavonoids, are important contributors to the antimicrobial properties of these extracts.

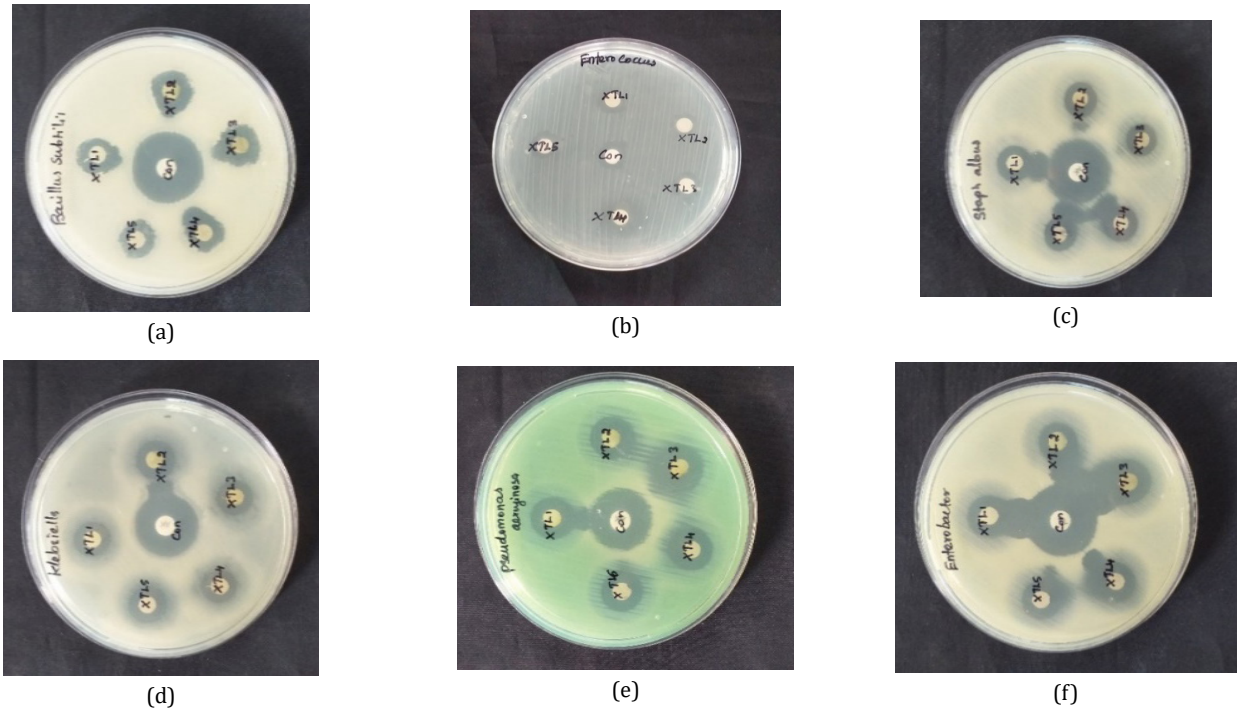


Fig. 5: Antibacterial activity of leaf extracts of *X. tomentosa* against (a) *Bacillus subtilis,* (b) *Enterococcus,* (c) *Staphylococcus albus*, (d) *Klebsiella,* (e) *Pseudomonas aeruginosa,* (f) *Enterobacter spp.*

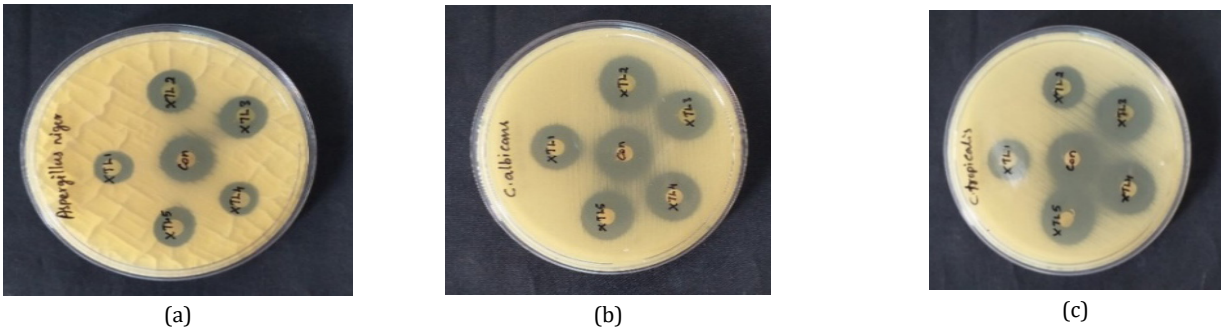


Fig. 6: Antifungal activity of leaf extracts of *X. tomentosa* (R), against (a) *Aspergillus niger,* (b) *Candida albicans*, (c) *Candida tropicalsis*

Methanol and acetone are effective against bacteria because they contain compounds like phenolics, flavonoids and terpenoids which damage the structure of microbial membranes; disrupt protein synthesis; and block the replication of DNA or RNA in the cells of bacteria. gram-positive bacteria are typically more susceptible to the effects of these phytochemicals than are gram-negative bacteria. This susceptibility difference is due to a thinner layer of peptidoglycan in the walls of Gram-positive bacteria, allowing for easier access into their interior compared to the thicker layers found in gram-negative cell walls (with their outer membrane limiting entry into the cytoplasm). Other Sapotaceae plants and extracts with high levels of polyphenolics demonstrate similar patterns of activity. Our data support earlier reports of broad-spectrum activity from polyphenolics. In addition, polar solvents are more effective than non-polar solvents at extracting antimicrobial agents from plant material. The comparison of antimicrobial activity is demonstrated in Fig. 7.

GC-MS Analysis

The use of GC-MS was applied to analyse the leaf extract of *X. tomentosa* for phytochemicals that could serve as bioactive compounds, as shown in Table 4. The identification of the phytochemical constituents cyclopentasiloxane and tetradecane, compounds that have previously been identified as exhibiting antimicrobial and cytotoxic activities, is likely a contributing factor to the demonstration of antibacterial activity in this

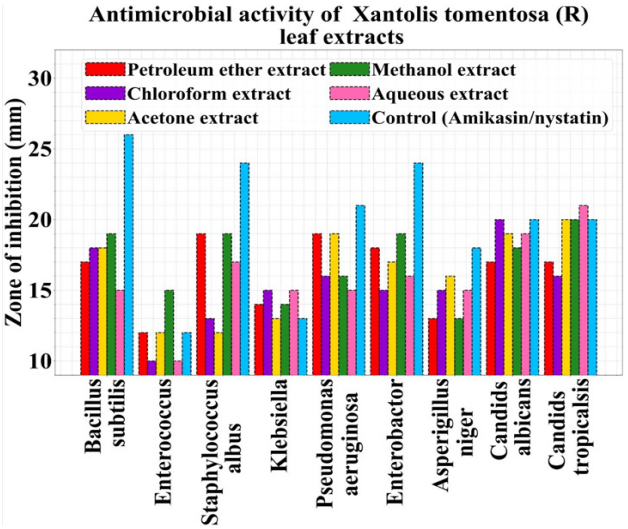


Fig. 7: Comparison of antimicrobial activity

study. Additionally, long-chain hydrocarbons nonadecane (C19H40) and 2,3,7-trimethyl decane (C15H32) are known to have antioxidant and antibacterial activities and correlate with the demonstrated scavenging capacity of free radicals in the antioxidant assays. In addition, bis(2-ethylhexyl) ester, 1,3-Benzenedicarboxylic acid and Bis(2-ethylhexyl) phthalate have demonstrated antiviral, antitumour and immunomodulatory properties, further suggesting potential uses for the extract in the development of therapeutic agents. Betulinaldehyde, a triterpene derivative, has also been documented to possess anticancer and antitumor activity, and an additional identified compound, 1,2-bis(trimethylsilyl)benzene, has demonstrated anti-inflammatory, antibacterial and antifungal effects, which help further establish the broad-spectrum antibacterial activity of the extract. In summary, the demonstrated presence of these bioactive molecules further demonstrates the potential medicinal relevance of *X. tomentosa* in the ethnopharmacological community. The biological activity demonstrated by the sample can be correlated through the GC-MS profile, which contains chemical evidence that correlates to the pharmacological properties of many of the compounds found in the sample. Long-chain hydrocarbons like nonadecane and 2,3,7-trimethyl decane show antioxidant and antimicrobial properties due to their interaction with the tricellular membrane structures of microorganisms and their ability to neutralize reactive metabolic products. Triterpenes, such as betulinaldehyde, have been reported numerous times to possess both anticancer and anti-inflammatory effects.[40] The neutral phthalate derivatives that were found in the extract also demonstrate antiviral and immunomodulatory effects.[41] The identification of these chemical class compounds provides support for the oxidative stress-reducing and microbial inhibitory effects identified from earlier studies and confirms the potential therapeutic properties of *X. tomentosa*.[42]

RSM Analysis

The experimental outcomes from the RSM optimization study are displayed in Table 5. Three independent variables were optimized, including solvent composition (A), extraction temperature (B), and extraction time (C), to study their interactive effects on the yield of phytochemicals (flavonoids, phenols, terpenoids) and their related biological activities (DPPH scavenging, NO scavenging, and antimicrobial efficiency). The high solvent compositions (70–80%) and high temperature (60–70°C) generally produced a higher yield of phytochemicals with increased bioactivity. For example, runs 2 and 9 had the highest flavonoid yield (11.8 mg/g and 11.4 mg/g, respectively) and phenolic yield (0.98 mg/g and 0.93 mg/g, accordingly) with the highest bioactivity (i.e., antioxidant yields (up to 83.2%) and maximum antimicrobial inhibition zones (up to 20.14 mm)). On the other hand, the lower solvent compositions (40–50%) and lower temperature (i.e., runs 1 and 8) produced significantly lower phytochemical yields with lower antioxidant/antimicrobial activity. This clearly supports that solvent polarity and extraction temperature are the two most

Table 4: Bioactive compounds identified using GC-MS analysis and their biological activity

Compound name	*Retention time*	*Chemical formulae*	*Molecular weight*	*Biological activity*
Cyclopentasiloxane	10.05	$C_{10}H_{30}O_5Si_5$	370	Antimicrobial activity
Tetradecane	16.15	$C_{14}H_{30}$	198	Antimicrobial, cytotoxic activity
Nonadecane	20.25	$C_{19}H_{40}$	268	Antimicrobial, antioxidant, anticancer
2,3,7- trimethyl decane	20.26	$C_{13}H_{28}$	184	Antibacterial, antioxidant activity
Bis(2-ethylhexyl) phthalate	34.90	$C_{24}H_{38}O_4$	390	Antiviral, antioxidant, antitumour activity
1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	37.51	$C_{24}H_{38}O_4$	390	Anticancer, immunomodulatory activity.
Betulinaldehyde	38.09	$C_{30}H_{48}O_2$	440	Antitumour activity
1,2-Bis(trimethylsilyl)benzene	38.73	$C_{12}H_{22}Si_2$	222	Anti-inflammatory, antibacterial, antifungal activity.

Table 5: RSM experimental results

Run	*A:Solvent composition*	*B:Extraction temperature*	*C: Extraction time*	*TFC*	*TPC*	*TTC*	*DPPH radical scavenging activity*	*NO radical scavenging activity*	*Antimicrobial activity*
	%	°C	h	mg/g	mg/g	mg/g	%	%	mm
1	40	30	1	5.6	0.47	0.21	46.3	42.8	11.42
2	80	70	5	11.8	0.98	0.48	74.5	83.2	20.14
3	40	70	5	8.34	0.72	0.35	69.8	69.7	17.4
4	80	30	1	7.4	0.65	0.3	61.5	57.2	15
5	60	50	3	8.79	0.75	0.35	67.13	70.52	16.67
6	50	40	2	7.2	0.61	0.28	60.1	59.8	14.2
7	70	60	4	10.6	0.9	0.44	72.4	80.5	18.9
8	45	35	1.5	6.1	0.55	0.25	53.7	50.4	13
9	75	65	4.5	11.4	0.93	0.46	73.8	82.7	19.4
10	55	45	2.5	7.9	0.68	0.31	63.5	63.1	15.2
11	65	55	3.5	9.9	0.84	0.4	70.7	77.1	18.2
12	40	50	3	6.8	0.63	0.29	60.6	60.54	14.79
13	80	50	3	10.9	0.87	0.43	73	81.3	18.7
14	60	30	2	6.5	0.57	0.26	57.12	54.19	13.4
15	60	70	4	10.3	0.82	0.4	72	79.4	18.6
16	50	60	5	8.92	0.77	0.38	70	74.33	16.8
17	70	40	1.5	8.09	0.7	0.33	64.11	64.82	16.4
18	45	70	2.5	7.7	0.65	0.33	62.8	64	15.5
19	75	30	4	8.3	0.72	0.35	65.1	68.5	16
20	55	35	3	7	0.6	0.29	59.3	57.6	14.3
21	65	45	1	7.5	0.66	0.31	63.47	61	14.9
22	40	35	4.5	7.2	0.62	0.3	60.8	59.1	14.7
23	80	65	2	10.7	0.89	0.43	73.14	78.3	18.5
24	50	50	4	8.1	0.7	0.34	65.8	67	16.2
25	70	55	1	8.5	0.74	0.36	67.2	70.1	16.6
26	45	60	3.5	7.8	0.67	0.32	65.54	65.4	15.7
27	75	45	2	9.1	0.79	0.38	68.9	73	17
28	65	30	3	7.3	0.63	0.3	60	58.3	14.5
29	55	70	1.5	8.6	0.71	0.37	65.6	71.5	16.5
30	50	65	2.5	8	0.71	0.33	65.2	68.1	16.1

influential conditions, as these variables provide an appropriate release of phenolic compounds and flavonoids, which are direct contributors to strong antimicrobial and antioxidant effects. Collectively, the RSM results confirm that optimizing extraction conditions can greatly increase the therapeutic properties of the extracted *X. tomentosa* leaves.

RSM analysis showed that solvent concentration and temperature of extraction had the greatest impact on the yield and bioactivity of phytochemicals. Polarity increases the ability for the solvent to dissolve phytochemicals (enhanced solubility) and facilitates the movement of phytochemicals from the plant to the solvent (increased mass transfer). Using moderate heat to extract phytochemicals disrupts the plant tissue and helps to improve the diffusion of flavonoids, phenolics, and terpenoids from the plant into the solvent. The correlation between increased phytochemical concentration and

Table 6: ANOVA for the quadratic model

Response	*F-value*	*P-value*	*Model*	*Lack of fit*
TFC	78.39	< 0.0001	significant	Not significant
TPC	146.90	< 0.0001	significant	Not significant
TTC	122.66	< 0.0001	significant	Not significant
DPPH radical scavenging activity	188.40	< 0.0001	significant	Not significant
NO radical scavenging activity	71.21	< 0.0001	significant	Not significant
Antimicrobial activity	76.47	< 0.0001	significant	Not significant

greater antioxidant/antimicrobial activity supports the results obtained from other studies on medicinal plants.[43] The results obtained demonstrate that process optimization is essential and that extraction conditions can greatly increase the medicinal properties of *X. tomentosa.* ANOVA Analysis showed that the quadratic model had a statistically significant impact on all responses based on F-value and *p-value* (<0.0001) as presented in Table 6. Hence, the study indicates that solvent concentration, extraction temperature, and extraction time play a critical role in determining phytochemical content and bioactivity. In addition, the lack-of-fit values were not significant; indicating that the models developed fit the experimental data very well without unexplained variation. In simpler terms, this suggests that the developed models are valid, reliable, and appropriate for predicting the responses over the selected experimental ranges.

Table 7 illustrates the fit statistics from the models, providing additional evidence of the appropriateness of the RSM models. The R^2 values (0.96–0.99) show that the model accounts for more than 96% of the variation in each response. The adjusted R^2 values are not overly distant from the R^2 values, suggesting that concerns regarding overfitting are not an issue in the model. The predicted R^2 values matched well with the adjusted R^2 values, suggesting that the model has good predictive power for new experimental conditions (not used in the model development process). The adequate precision values (>4) for all responses indicated a good signal-to-noise ratio, which supports the conclusion that the model is highly appropriate to use in the exploration of the experimental design space. The low values for the standard deviation demonstrate carefulness in making and recording accurate and precise measurements during the laboratory studies. Overall, these statistical parameters confirm that the quadratic RSM model is robust, correct, and suitable for optimization of extraction conditions.

Regression Equation in Coded Form

$$TFC = +8.69 + 1.55\,A + 1.17\,B + 0.7917\,C + 0.4950\,AB - 0.0069\,AC + 0.0769\,BC + 0.0836\,A^2 - 0.3281\,B^2 - 0.2450\,C^2 \quad (6)$$

$$TPC = + 0.7437 + 0.1179\,A + 0.0854\,B + 0.0640\,C + 0.0251\,AB + 0.0008\,AC + 0.0023\,BC + 0.0100\,A^2 - 0.0461\,B^2 - 0.0062\,C^2 \quad (7)$$

$$TTC = + 0.3528 + 0.0606\,A + 0.0478\,B + 0.0362\,C + 0.0094\,AB + 0.003\,AC - 0.0124\,BC + 0.0050\,A^2 - 0.0169\,B^2 + 0.0073\,C^2 \quad (8)$$

$$\text{DPPH radical scavenging activity} = + 67.15 + 5.39\,A + 4.98\,B + 3.80\,C - 0.1922\,AB - 2.14\,AC - 0.4354\,BC - 0.4863\,A^2 - 2.82\,B^2 - 0.3061\,C^2 \quad (9)$$

$$\text{NO radical scavenging activity} = + 70.37 + 8.46\,A + 8.03\,B + 5.78\,C + 0.1117\,AB + 0.0295\,AC - 1.87\,BC - 0.6336\,A^2 - 4.22\,B^2 - 0.1893\,C^2 \quad (10)$$

$$\text{Antimicrobial activity} = + 16.55 + 1.77\,A + 1.63\,B + 1.11\,C + 0.1688\,AB - 0.2740\,AC + 0.0650\,BC + 0.0968\,A^2 - 0.5895\,B^2 - 0.1889\,C^2 \quad (11)$$

The regression equations above (Equations 6-11) describe the interaction among the independent variables (A, B and C) and the identified output variables. The positive coefficients for A, B, and C indicate that elevating their level will increase the extraction yield of phytochemicals and enhance antioxidant, as well as antimicrobial, characteristics. Interaction terms (AB, AC, BC) and quadratic terms (A^2, B^2, C^2) describe how variables interact and how the non-linear effects on responses. Among the

Table 7: Fit statistics

Response	*Std. Dev.*	*R^2*	*Adjusted R^2*	*Predicted R^2*	*Adeq precision*
TFC	0.3158	0.9724	0.9600	0.9117	38.4567
TPC	0.0176	0.9851	0.9784	0.9651	52.7606
TTC	0.0103	0.9822	0.9742	0.9467	48.6748
DPPH radical scavenging activity	0.8322	0.9883	0.9831	0.9666	58.9362
NO radical scavenging activity	2.11	0.9697	0.9561	0.9160	36.6294
Antimicrobial activity	0.4079	0.9718	0.9591	0.9173	38.3330

independent variables, solvent concentration (A) and extraction temperature (B) showed the most significant overall effect. All models showed statistical significance in the ANOVA framework and were used to generate response surface plots and optimization results.

Sensitivity Analysis

The sensitivity analysis presented in Fig. 8(a–c) illustrates how each extraction factor impacts the yield of specific phytochemicals. The TFC in Fig. 8(a) exhibited a significant positive response when increasing the concentration of the extraction solvent (Factor A). In particular, it can be hypothesized that the specific relevance of solvent polarity is specifically relevant, as the polarity of the extraction solvent increases, the solubility of the flavonoid compounds, and thereby extraction productivity increases. Fig. 8(b) suggests that both the solvent concentration (A) and the extraction time (C) were essential contributors to the total phenol content, meaning that phenolic substances appear to require both sufficient solvent strength and time to diffuse from the tissue effectively. Furthermore, Fig. 8(c) displays the total terpenoid yield factor as being affected predominantly by the solvent concentration (A), with some contribution from the temperature (B), consistent with the semi-nonpolar nature of terpenoids, which increase in recovery when interacted with the solvent favorably. Overall, Fig. 5 indicates that among the three phytochemical groups, the composition of the solvent is the most important extraction factor influencing the extraction efficiency.

Fig. 9(a–c) illustrates the perturbation plots, which display the sensitivity of biological activities to variations in extraction conditions. The solvent concentration and the extraction time increased the DPPH radical scavenging activity, as shown in Fig. 9(a), which indicates that antioxidant compounds such as phenols and flavonoids are released more easily during ideal extraction conditions. The nitric oxide scavenging activity (NO) shown in Fig. 9(b) was driven most prominently by temperature. As the extraction was heated to moderate levels, the effects of moderate temperature helped break down the cell walls of the extract and improved the diffusion of bioactive molecules, without denaturing them before extraction. In Fig. 9(c), antimicrobial activity displayed a strong positive response to increased solvent concentration, demonstrating that polar solvents are more efficient at extracting antimicrobial phytochemicals. The extraction time also provided a supportive enhancement. Overall, perturbation analysis confirms that solvent concentration is the most critical factor influencing biological activity, followed by extraction temperature and time.

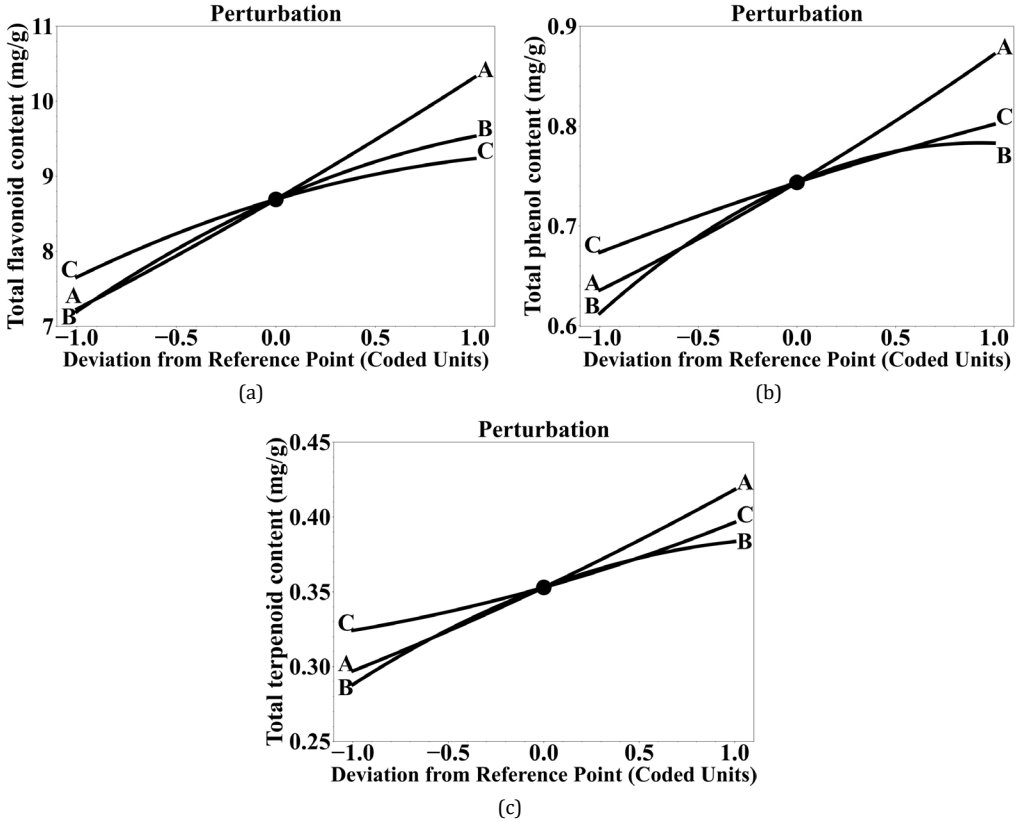


Fig. 8: Perturbation plots of (a) TFC, (b) TPC and (c) TTC

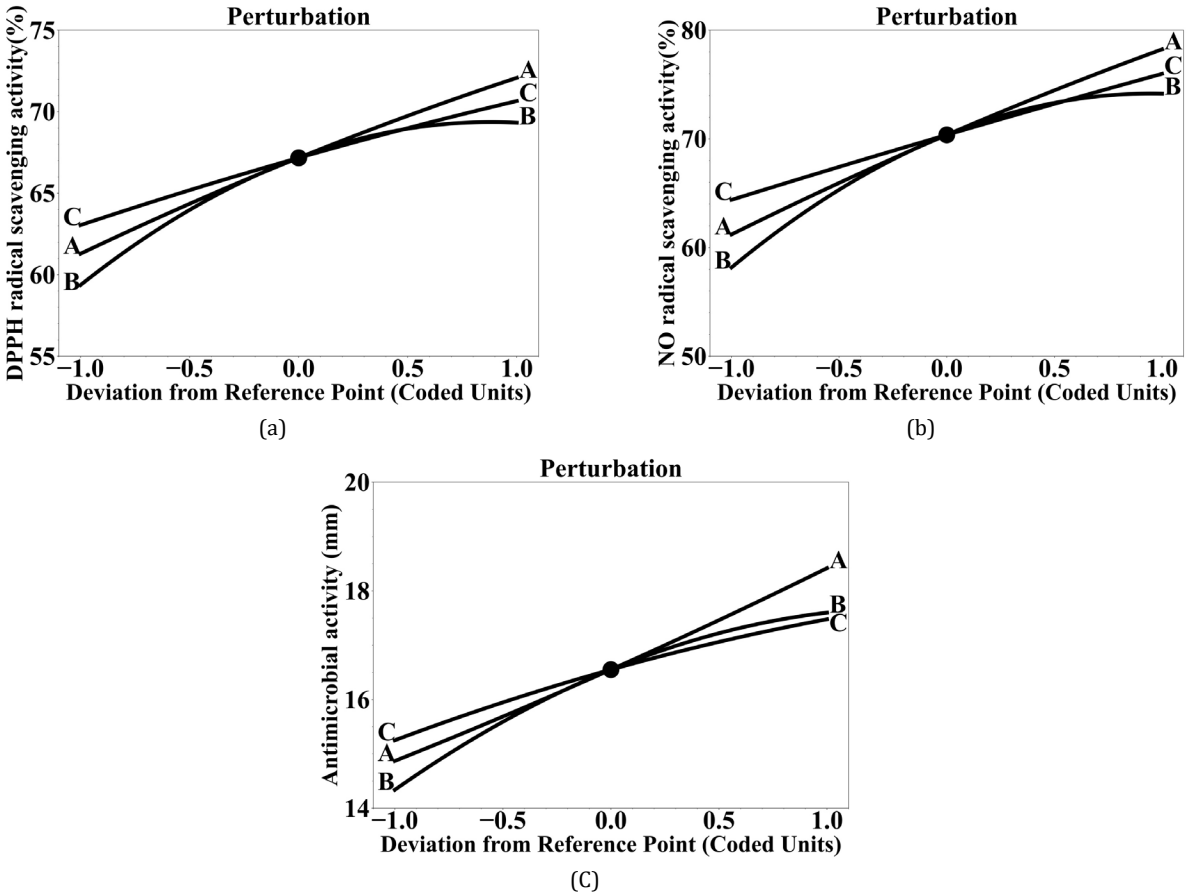


Fig. 9: Perturbation plots of (a) DPPH, (b) NO, and (c) Antimicrobial activity

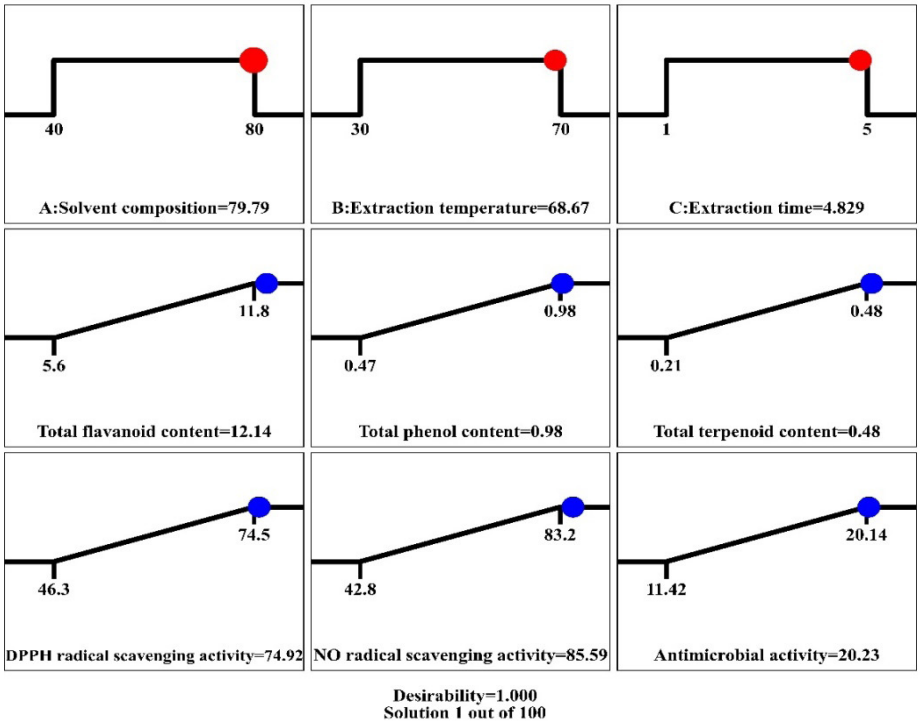


Fig. 10: optimization plot

Desirability analysis

Fig. 10 depicts the desirability optimization plot, which considers all response variables to select conditions that result in the best extraction potential for phytochemical yield and biological activities, at the same time. The desirability plot shows that, when combined, the output approaches 1.000; the experimental data fit the model well. The predicted optimal extraction parameters were approximately 79.79% solvent, 68.67°C extraction temperature, and 4.83 hours of extraction time. When these parameters were optimized, the model predicted maximum yields of total flavonoids (12.14 mg/g), total phenols (0.98 mg/g), and total terpenoids (0.48 mg/g) and maximized DPPH (74.5%) and NO (83.2%) radical scavenging activities, and a zone of inhibition for antimicrobial activity of 20.14 mm. Overall, the desirability plot indicated that selected conditions were possibly effective and statistically valid for maximum extraction efficiency and biological efficacy.

Conclusion

X. tomentosa leaves were successfully extracted and analyzed to evaluate their phytochemical composition and biological activities. Active compound recovery and the functional properties were significantly improved after the extraction conditions were optimized. The best settings for bioactive yield were temperature (50°C), time 40 minutes, and the concentration of the solvent, 75% methanol.

1. The TFC was improved to 11.8 mg/g while the maximum TPC was 0.98 mg/g under the optimum conditions.
2. The antioxidant activity showed high DPPH radical inhibition rates of 74.5 – 83.2%, suggesting high free-radical scavenging capability.
3. The antimicrobial activity was measured as 20.14 mm inhibition zones for the pathogen studied, suggesting anti-pathogen activity towards the microorganisms tested.
4. The most interesting compounds detected by GC-MS analysis included nonadecane, bis (2-ethylhexyl) phthalate, and betulinaldehyde, which show antimicrobial, anticancer, anti-inflammatory, and antioxidant activity.

Limitations and Future Scope

The biological potential of the extracts was significant; however, it is important to note that the developed investigation was limited to the *in-vitro* profiling of the chemical composition. Future research will include isolation and characterization of compounds from the extracts of *X. tomentosa* that have demonstrated bioactivity, as well as elucidation of the mechanisms by which these compounds exert bioactivity *in-vitro* and the in vivo toxicology studies and pharmacology. Following this, formulation development will be necessary along with clinical trials in order to establish the ability of products made from *X. tomentosa* to serve as safe and effective natural agents in a clinical setting, as well as possible sources of new pharmacological agents.

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HOW TO CITE THIS ARTICLE: Vismaya MR, Nevaditha NT. Investigating Bioactive Compound Extraction and Evaluation of *Xantolis tomentosa* based on Response Surface Methodology. Int. J. Pharm. Sci. Drug Res. 2026;18(2):71-86. DOI: 10.25004/IJPSDR.2026.180201