Phytochemical analysis, in vitro antioxidant and antimicrobial activities of male flower of Juglans regia L.

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ABSTRACT

The male flower of Juglans regia L., were investigated for its in vitro antioxidant activity, antimicrobial activity, and chemical constituents. The antioxidant activity showed that the methanol extract of J. regia male flower (MEJR) had highest scavenging potential than the other solvents (ethanolic = EEJR and aqueous = AEJR). The antimicrobial activity showed that Staphylococcus aureus and Escherichia coli were the most sensitive organisms and significant activity was also recorded against both the fungal strains tested, with highest activity against Candida albicans. Totally, 26 constituents were identified by high-resolution-liquid chromatography-mass spectrometry analyses from which seven compounds were identified first time from the extract.

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KEYWORDS

Antimicrobial; Antioxidant; FTIR; Juglans regia L; LCMS

Introduction

The Juglandaceae family includes several genera among which Juglans genus is the important representative, with 7–45 species. Among these Juglans regia L. is one of the premium tree traditionally cultivated for its valuable wood and fruits. The seed is a nut of high economic interest to the food industry and is globally popular and valued for its nutritional, health, and sensory attributes. The vast biodiversity of Himalaya provides this royal species mostly in the Kashmir region, growing up to 25–35 m.

Juglans regia is considered to treat a variety of health complaints traditionally, including Cancer, Inflammation, Diabetes, Antiradicalar, Hyperhidrosis, Antidiarrheic, Prostate, Antiradicalar, and Cardiovascular disorders. However, researchers investigated that almost all parts of the plant are important against different health disorders as well as for preservation of food grains. The extracts from J. regia nut inhibited oxidative damages, inflammation, tumor growth, antiwrinkle, and photoaging. Kernels as a dietary food, against diabetes, hypoxia, some skin diseases, and inflammation, leaves as antidiarrheals, anthelmintic, deurletive, and also mixed with stored-grains as an insecticide and fungicide. Stem bark as an astringent, anthelmintic, deurletive, bactericide, diuretic, digestive, laxative, stimulant, detergent, and insecticidal. Juglans regia L. shell is reported for polishing gun-casings, jewelry, and metal material and is used as media to separate water and crude oil.

Juglans regia L. is a good source of flavonoids, Polyphenols, flavonols, carbohydrates, fatty acids, cardiac glycosides, steroids, minerals, tannins, protein, dietary fiber, melatonin, plant sterols, α-tocopherol, folate, tannins, vitamin A and C, and vitamin E family compound. Several studies demonstrated the antimicrobial activity of phenolic extracts, making them as best substitute to antibiotics and food preservatives. Juglans regia L. is a natural product of high economic interest to the food industry and is very popular and largely consumed as royal food globally and valued for its

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nutritional, health, and sensory attributes.\cite{1} There is an extended interest in using natural anti-
microbial compounds, due to the increasing resistance to antibiotics.\cite{24} Although all parts of this
valuable plant has been investigated for different biological properties, no study regarding the
phytochemical analysis, antioxidant and antimicrobial activity has been reported yet for the male
flower of \textit{J. regia} L. from Himalayan region. The objective of this study is thus to evaluate the
phytochemical analysis, antioxidant, and antimicrobial activity of male flower of \textit{J. regia} L. and its
further utilization in food products.

\section*{Materials and methods}

\subsection*{Reagents and instruments}

2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), \(\alpha,\alpha\)-Diphenyl-2-picrylhydrazyl (DPPH), 2,2\-' azino-bis(3-
ethylbenzothiazoline-6-sulfonic acid) (ABTS), ascorbic acid (ABA), sodium nitrite, aluminum chloride,
metallic acid, and quercitin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo.,
USA). All organic solvents were of analytical grade (Merck, Darmstadt, Germany), a Multimode
reader (Tecan, Austria) were used for the determination of antioxidant and antimicrobial activities.
Spectrum RX1 FT-IR spectrophotometer (Perkin Elmer, USA) was used for FT-IR spectra recording
and liquid chromatography/mass spectrometry (UHPLC-PDA-Detector Mass spectrometer (HR-
LCMS 1290 Infinity UHPLC System, 1260 infinity Nano HPLC with Chipcube, 6550 iFunnel QTOFs), Agilent Technologies, USA) were used for polyphenolics analysis and identification.

\subsection*{Collection and preparation of plant extracts}

The male flower part of \textit{J. regia} L. (locally referred to as \textit{Doon Fidin}) were collected from the Indian
Himalayan region of Jammu and Kashmir during the flowering stage (April 2014), at an altitude
33.7167°N 74.8333°E. The plant materials were identified by Dr. D. Kumarasamy, the designated
plant taxonomist of the Department of Botany, Annamalai University, with herbarium voucher no.
\textbf{ABH-2023}. The plant material was shade dried and powdered by using electric grinder. Plant
material was soaked into solvents (95% methanol and ethanol and water) in a ratio of 1: 6 (w/v)
for 48 h at 20°C with vigorous shaking. The extract was subjected to evaporation under vacuumed
pressure and residual material was considered as source of crude extract and was stored at 4°C for
further analysis. The extraction yield has been calculated by fallowing the method of\cite{25} directly by
using the formula

\begin{equation}
\text{Extraction yield} \, (\%) = \frac{\text{weight of crude extract}}{\text{weight of the original sample}} \times 100
\end{equation}

\subsection*{Antioxidant assays}

\textbf{ABTS radical scavenging assay}

ABTS method measures the capacity of different compounds to scavenge the 2,2-azino-bis-3-
ethylbenzothiazoline-6-sulphonic acid radical cation (ABTS\(^{+}\)).\cite{26} The antioxidant activity was
measured in a reaction mixture containing 0.5 mL of 15 \(\mu\)M \(\text{H}_2\text{O}_2\), 0.5 mL of 7 mM ABTS, and
50 mM sodium phosphate buffer, pH 7.5. The absorbances were recorded by spectrophotometer at
734 nm and were compared with standard ascorbic acid. \(\text{IC}_{50}\) value is the concentration of sample
required to inhibit 50% of ABTS\(^{+}\) production. The percentage of ABTS radical scavenging was
calculated as given below:

\begin{equation}
\% \text{scavenging} \, [\text{ABTS}^{+\prime}] = \frac{A_0 - A_1}{A_{0x}} \times 100
\end{equation}

Where \(A_0\) was the absorbance of the control and \(A_1\) was the absorbance in the presence of the
sample of ascorbic acid (ABA).
**Ferric reducing antioxidant power (FRAP) assay**

The FRAP assay was performed using a TPTZ solution. The working solutions were prepared by mixing 25 mL of acetate buffer (pH 3.6, 300 mM), 2.5 mL of TPTZ solution (10 mM), and 2.5 mL of FeCl₃ · 6H₂O solution (20 mM), and were kept at 37°C. The sample solutions with different concentrations (mg/mL) were then mixed with the working solutions and were incubated under darkness at 37°C for 30 min. The absorbance was measured at 650 nm. The antioxidant activities were expressed as µmol/L FeSO₄ equivalent/mg extracts, called FRAP values. A higher FRAP value corresponds to a greater antioxidant activity.

**DPPH radical scavenging capacity**

Free radical scavenging property of different extracts of *J. regia* male flower against DPPH• (2, 2-diphenyl-1-picrylhydrazyl) was determined spectrophotometrically by the method of. DPPH• is a stable free radical, becomes a stable diamagnetic molecule when gets reduced. DPPH• donate hydrogen when reacts with an antioxidant and gets reduced, this change in color was measured (from deep violet to light yellow) which is directly proportional to the amount and nature of radical scavenger present.

**Antimicrobial activity**

**Microorganisms**

The antimicrobial activity of male flower of *J. regia* was tested against two Gram positive bacterial strains viz. *Staphylococcus aureus* and *Bacillus subtilis*, two Gram negative bacterial strains viz. *Escherichia coli* and *Proteus vulgaris*, and against two fungal strains viz. *Candida albicans* and *Candida glabrata*. The microorganisms were obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, India. The stock cultures were maintained on Muller–Hinton Agar (for bacteria) and Sabouraud dextrose agar medium (for fungi) at 4°C.

**Disc diffusion assay and determination of minimum inhibitory concentration (MIC)**

Antimicrobial activity of different extracts of male flower of *J. regia* was determined by using disc diffusion method with little modifications. The susceptibility tests were performed on Muller–Hinton Agar (bacteria) and Sabouraud Dextrose Agar (fungi). 50, 25, and 12.5 mg (diluted with mg/mL 5% dimethyl sulfoxide (DMSO)) of all extracts were impregnated on the filter paper discs (6 mm) and used for the study. Ciprofloxacin (5 mg/disc) for bacteria and Amphotericin B (20 mg/disc) for fungi were used as positive reference standards to determine the sensitivity of the tested strains and 5% DMSO was used as blind control. Finally, the inoculated plates were incubated at 37°C for 24 h (for bacteria), 28°C for 48 h (for Candida) and the inhibition zones were observed including the diameter of the disc (6 mm).

MIC of the male flower of *J. regia* was tested by the twofold serial dilution method with little modifications. The extracts were dissolved in 5% DMSO to obtain 100 mg/mL stock solution. 0.5 mL of stock solution was incorporated into 0.5 mL of Mueller Hinton broth (bacteria) to get the concentration of 100 mg/mL and serially diluted to achieve 50, 25, and 12.5 mg/mL. Fifty microliters of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms, without extracts and 5% DMSO was used as blind control. The culture tubes were incubated at 37°C for 24 h. The lowest concentrations, which did not show any growth of tested organisms after macroscopic evaluation was determined as MIC.

**Phytochemical investigation**

**Total phenolic content (TPC)**

TPC of MEJR, EEJR, and AEJR were analyzed by using Folin–Ciocalteu method followed by with little modifications. 0.5 mL of extracts (0.5 mg/mL) were diluted with 6 mL of double-distilled water,
then mixed with 0.5 mL of 1N Folin–Ciocalteu’s reagent and were allowed to settle for 5 min. consequently 2 mL of 20% Na₂CO₃ solution were supplemented. Absorbance was measured at 750 nm by using UV–VIS spectrophotometer (Tecan, Austria), after the incubation of 1 h at RT. Gallic acid has been used as standard. Total content of phenolics were expressed as mg gallic acid equivalents/100 g DW.

**Total flavonoids content (TFC)**

TFC of MEJR, EEJR, and AEJR were determined on the basis of formation of flavonoid-aluminum complex by using spectrophotometric method of[32] with little modifications. 0.5 mL of 2% AlCl₃ (aluminum chloride) with methanol were added to 0.5 mL of extract. Absorbances were measured at 430 nm after the incubation of 15 min at room temperature. Quercitin has been used as standard. The total content of flavonoids was expressed as mg quercitin equivalents/100 g DW. The values were presented as means of triplicate analyses.

**Fourier transform infrared (FT-IR) spectroscopy analysis**

FT-IR spectra were recorded by using Spectrum RX1 FT-IR spectrophotometer (Perkin Elmer, USA) working in 4000–400 cm⁻¹ region, Outfitted with a KBr beam splitter, DTGS detector and Nichrome source. Total of 100 scans were acquired for Final spectrum with 4 cm⁻¹ resolution. For the preparation of translucent sample discs, the dried powdered extract was encapsulated in KBr. pellet.

**High resolution-liquid chromatography-mass spectrometry analysis (HR-LCMS)**

The HR-LCMS analysis of MEJR was analyzed by with some modifications, by using UHPLC-PDA-Detector Mass spectrometer (HR-LCMS 1290 Infinity UHPLC System, 1260 Infinity Nano HPLC with Chipcube, 6550 iFunnel QTOFs), Agilent Technologies, USA. For chromatographic separation, an Agilent 1200 Series HPLC system (Agilent Technologies, USA) equipped with a binary gradient solvent pump, HiP Sampler, column oven and MS Q-TOF with Dual AJS ES Ion Source. Samples were separated on SB-C18 column (2.1 × 50 mm, 1.8-particle size; Agilent Technologies, USA) maintained at 25°C. The solvents used were: water containing 0.1% HCOOH and methanol containing 0.1% HCOOH. The following gradient elution program at a flow rate of 0.4 mL min⁻¹ was applied. MS detection was performed in MS Q-TOF Mass spectrometer (Agilent Technologies). The identified constituents were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the corresponding standards. Linearity ranges for calibration curves were specified.

**Statistical analysis**

All of the examinations were executed in triplicate, and the values were expressed as the mean ± standard deviation. The results were evaluated through analysis of variance (ANOVA) with Duncan’s multiple range tests (p < 0.05) using SPSS.

**Results and discussion**

**Antioxidant activity of JR**

To evaluate the antioxidant activities of three different extracts of male flower of *J. regia* L., DPPH, ABTS, and FRAP assay were analyzed as shown in Table 1. The DPPH, ABTS, and FRAP assays are based on electron transfer between sample and the reagent radical and are measured by evaluating their color changes spectrophotometrically. All three extracts of *J. regia* showed the significant free radical scavenging activity against DPPH, BTS, and FRAP assay, comparing with the high
antioxidant effect of ascorbic acid. However, MEJR showed the highest antioxidant activity having IC$_{50}$ value of 66.80 ± 2.13 for DPPH, 53.95 ± 6.46 for ABTS, and also FRAP value of 43.60 ± 4.83 µmol/L FeSO$_4$/mg extract as compared with EEJR having IC$_{50}$ value of 75.17 ± 4.43 for DPPH, 63.40 ± 5.73 for ABTS and FRAP value of 54.35 ± 3.12 µmol/L FeSO$_4$/mg extract, followed by AEJR having IC$_{50}$ value of 76.76 ± 3.75 for DPPH, 64.30 ± 7.23 for ABTS and FRAP value of 52.70 ± 2.90 µmol/L FeSO$_4$/mg extract. The free radical scavenging activity of different extracts are comparable with previously reported studies on leaves, bark, stem, and nuts of *J. regia* by using DPPH, ABTS, and FRAP assays.\[8,22,33–36\]

The results of the total phenolic content evaluated using Folin-Ciocalteu method, are shown in Table 1 to support the antioxidant activity of the male flower of *J. regia*. MEJR showed the higher TPC (129.76 ± 3.11 mg/g DM) as compared to EEJR (124.12 ± 2.45 mg/g DM) and AEJR (122.1 ± 2.80 mg/g DM), which is similar with previously reported TPC (116.39 ± 5.63 and 92 ± 1.40 mg GAE/g) of *J. regia* nuts in methanolic and petroleum ether extracts, respectively.\[8\]

Flavonoids are secondary metabolites, with several health benefits such as antioxidant, anti-inflammatory, and antimicrobial activities.\[37\] Rutin, quercetin, Gallic acid, and kaempferol are major flavonoids reported previously in *J. regia*.\[38,39\] The TFC values in the present study were highest in MEJR (144.62 ± 2.40 mg/g) followed by EEJR (137.81 ± 3.28 mg/g) and AEJR (131.79 ± 4.69 mg/g) of *J. regia* nuts in methanolic and ethyl acetate extract of Shell of *J. regia* where TFC was 48.90 ± 0.7 and 80.40 ± 0.55 mg QEs/g extract, respectively.\[40\]

### Table 1. Antioxidant activity of different extracts of male flower of *J. regia* L.

| Sample | Yield (%) | DPPH$^*$ | ABTS$^{+}$ | FRAP (µmol/L FeSO$_4$/mg extract) | TFC (µmol/L FeSO$_4$/equiv/mg extracts) | TPC (mg/g DM) |
|--------|-----------|---------|------------|---------------------------------|----------------------------------|-------------|
| MEJR   | 12.3      | 66.80 ± 2.13$^b$ | 53.95 ± 6.46$^b$ | 43.60 ± 4.83$^b$ | 144.62 ± 2.40$^c$ | 129.76 ± 3.11$^c$ |
| EEJR   | 8.8       | 75.17 ± 4.43$^c$ | 63.40 ± 5.73$^c$ | 54.35 ± 3.12$^c$ | 137.81 ± 3.28$^b$ | 124.12 ± 2.45$^b$ |
| AEJR   | 7.9       | 76.76 ± 3.75$^c$ | 64.30 ± 7.23$^c$ | 52.70 ± 2.90$^c$ | 131.79 ± 4.69$^a$ | 122.1 ± 2.80$^a$ |
| ABA    | —         | —       | —          | —                              | —                                | —           |

DPPH radical scavenging activities; ABTS radical scavenging activities; Ferric reducing antioxidant power (FRAP) and Total flavonoid contents (TFC) and Total phenolic contents (TPC) of male flower of *J. regia* L. Samples (µmol/L FeSO$_4$ equivalent/mg extract). Data are the mean of three independent analyses of each representative variety (mean ± SD; n = 3).

$a, b, c$ Data are shown as mean ± SD, n = 3. Data with different super script letters in the same column are significantly different (p < 0.05).

### Antimicrobial activity

The antimicrobial potential of male flower of *J. regia* against two Gram positive and Gram negative bacteria and two fungi were evaluated by using disc diffusion method and determination of minimum inhibitory concentration and the results are presented in Table 2. The results revealed that the male flower of *J. regia* showed significant antimicrobial activity against all the bacterial and fungal strains tested and Gram positive bacteria were more susceptible than Gram negative bacteria and fungi. The mean zone of inhibition produced by all the extracts ranged from 9.28 ± 0.5 to 46.13 ± 4.1 mm and the MIC value were between 2.6 and 4.7 mg/mL. The MEJR showed highest antimicrobial activity with the highest mean zone of inhibition (46.13 ± 4.1 mm) and lowest MIC (2.6 mg/mL) values against *S. aureus* followed by EEJR against *E. coli* (39.19 ± 4.3 mm; MIC = 3.9) and AEJR against *B. subtilis* (39.15 ± 2.9 mm; MIC = 3.0). MEJR also showed the highest mean zone of inhibition against *E. coli* (44.7 ± 3.2 mm; MIC = 3.4); and *P. vilgaris* (43.12 ± 1.8 mm; MIC = 3.0 µg/mL). The highest antifungal activity was also observed in MEJR against *C. albicans* (40.57 ± 3.7 mm) and *C. globarata* (39.4 ± 2.5) followed by EEJR against *C. albicans* (33.73 ± 3.52 mm) and AJR against (36.2 ± 1.8).
| Name of the organism | MEJR (12.5mg/mL) | MEJR (25mg/mL) | MEJR (50mg/mL) | EEJR (12.5mg/mL) | EEJR (25mg/mL) | EEJR (50mg/mL) | AEJR (12.5mg/mL) | AEJR (25mg/mL) | AEJR (50mg/mL) | Control drug | MIC (mg/mL) |
|----------------------|------------------|----------------|----------------|------------------|----------------|----------------|------------------|----------------|----------------|--------------|--------------|
| **Grampositive bacteria** | | | | | | | | | | | | |
| Staphylococcus aureus | 15.62 ± 1.32 | 29.50 ± 2.01 | 46.13 ± 4.21 | 11.43 ± 0.70 | 19.35 ± 1.35 | 34.73 ± 1.95 | 10.29 ± 1.10 | 17.05 ± 2.45 | 36.26 ± 4.18 | 41.1 ± 3.75 | NT | 2.6<sup>a</sup> 4.5<sup>b</sup> 4.5<sup>c</sup> |
| Bacillus subtilis | 13.28 ± 2.15 | 24.3 ± 1.35 | 42.2 ± 2.56 | 10.9 ± 1.03 | 21.56 ± 3.4 | 33.8 ± 2.22 | 7.46 ± 0.7 | 18.6 ± 3.1 | 39.1 ± 2.9 | 44.3 ± 2.53 | NT | 3.0<sup>a</sup> 5.3<sup>b</sup> 4.7<sup>c</sup> |
| **Gramnegativ bacteria** | | | | | | | | | | | | |
| Escherichia coli | 19.35 ± 2.00 | 31.5 ± 1.4 | 44.7 ± 3.2 | 14.2 ± 1.3 | 25.75 ± 2.4 | 39.19 ± 4.3 | 11.3 ± 0.16 | 22.6 ± 1.8 | 38.4 ± 3.2 | 42.6 ± 3.82 | NT | 3.4<sup>a</sup> 3.0<sup>b</sup> 4.7<sup>c</sup> |
| Proteus vulgaris | 16.17 ± 1.1 | 29.79 ± 3.6 | 43.12 ± 1.8 | 11.3 ± 0.8 | 21.2 ± 3.5 | 37.69 ± 2.1 | 9.28 ± 1.3 | 17.4 ± 2.7 | 37.3 ± 2.19 | 40.24 ± 3.5 | NT | 3.0<sup>a</sup> 4.0<sup>b</sup> 4.0<sup>c</sup> |
| **Fungi** | | | | | | | | | | | | |
| Candida albicans | 18.3 ± 2.7 | 27.2 ± 1.4 | 40.57 ± 3.7 | 12.65 ± 1.8 | 24.16 ± 1.31 | 37.73 ± 3.52 | 7.62 ± 0.6 | 17.8 ± 3.25 | 31.7 ± 3.7 | 37.14 ± 2.18 | NT | 2.5<sup>a</sup> 3.0<sup>b</sup> 3.0<sup>c</sup> |
| Candida glabrata | 15.2 ± 1.9 | 26.8 ± 3.6 | 39.4 ± 2.5 | 10.3 ± 0.9 | 24.12 ± 0.96 | 33.9 ± 1.23 | 9.4 ± 1.4 | 19.35 ± 2.1 | 36.2 ± 1.8 | 39.21 ± 4.74 | NT | 3.0<sup>a</sup> 4.0<sup>b</sup> 4.0<sup>c</sup> |

MIC: minimum inhibitory concentration; NT: not tested. (X = Mean zone of three assays. Y = Inhibition zones including the diameter of the disc (6 mm)).

<sup>a, b, c</sup> Data are shown as mean ± SD, n = 3. Data with different super script letters in the same column are significantly different (p < 0.05).
Recently, Farooqui et al.\textsuperscript{[41]} found the bark of \textit{J. regia} against 15 bacteria’s (\textit{viz}, \textit{Staphylococcus aureus}, \textit{Streptococcus pyogenes}, \textit{Enterobacter cloacae}, \textit{Citrobacter freundii}, etc.) with MIC ranging from 0.31 to >5 (mg/mL). Zakavi et al. (2013)\textsuperscript{[42]} reported that the ethanolic and aqueous extracts of \textit{J. regia} bark against some oral Bacteria (\textit{Staphylococcus aureus}, \textit{Streptococcus sanguis}, \textit{Streptococcus salivarius}, and \textit{Streptococcus mutans}), having MIC up to 5 mg/mL. Cruz-Vega et al. (2008)\textsuperscript{[43]} also found leaves and bark as the active aerial part of \textit{J. regia} against microbes with MICs ranging from 100 to 125 μg/mL. Noumi et al. (2010)\textsuperscript{[44]} reported the antibacterial effect of bark of \textit{J. regia} L. with MIC ratio ranging from 0.006 to 3.125 (mg/mL). The differences registered between our results and previously reported data could be attributed to the extraction procedure, the plant origin, the tested microorganisms, and the size of the inoculums. The results of antifungal activity of different extracts of \textit{J. regia} L., is comparable with the results of Pereira et al., 2007,\textsuperscript{[45]} Oliveira et al., 2007,\textsuperscript{[46]} and Sytykiewicz et al., 2015\textsuperscript{[47]} screened leaves and green husks of \textit{J. regia} from Portugal and Poland, against \textit{Candida spp.} with significant activity.

\textbf{FT-IR spectral analysis}

FT-IR spectral analysis of MEJR revealed the occurrence of various functional groups in it. Spectral data confirmed the existence of bioactive functional groups like alkanes (C–H stretch, C–H rock), alkenes (–C = C– stretch, = C–H bend), alkyynes (–C ≡ C–H: C–H stretch), 1º, 2º amines and amides (N–H stretch), α, β-unsaturated aldehydes and ketones (C = O stretch), 1º amines (N–H bend), aromatics (C–C in–ring), aliphatic amines (C–N stretch), and alkyl halides (C–Cl stretch). IR absorption frequencies and the representative spectra are shown in Table 3 and Fig. 1, respectively. The analyzed functional groups give the probable identification of compounds present in the extract and support the data analyzed by HR-LCMS.

\begin{table}[h]
\centering
\caption{Major bands observed in the FT-IR spectra of MEJR.}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{S. no} & \textbf{Frequency (m}^{-1}\text{)} & \textbf{Bond} & \textbf{Functional groups} & \textbf{Probable Phytochemical} \\
\hline
1 & 720.22 & C–H rock, C–Cl stretch, C–H oop, N–H wag & Alkanes, alkyl halides, aromatics, 1º, 2º amines & Alkaloids, flavonoids, tannins, poly phenols, carboxylic acid etc., containing phytochemicals \\
2 & 831.74 & C–Cl stretch, C–H oop, N–H wag, = C–H bend & alkyl halides, aromatics, 1º, 2º amines, alkenes & \\
3 & 910.19 & N–H wag, = C–H bend, O–H bend & aromatics, alkenes, carboxylic acids & \\
4 & 1057.79 & C–N stretch & aliphatic amines & \\
5 & 1164.65 & C–H wag(–CH2X), C–O stretch, C–N stretch & alkyl halides, alcohols, carboxylic acids, esters, ethers, aromatic amines & \\
6 & 1197.18 & C–H wag(–CH2X), C–O stretch, C–N stretch & alkyl halides, alcohols, carboxylic acids, esters, ethers, aromatic amines & \\
7 & 1244.85 & C–N stretch, C–H wag(–CH2X), C–O stretch & aromatic amines, alkyl halides, alcohols, carboxylic acids, esters, ethers, aromatic amines & \\
8 & 1277.53 & C–N stretch, C–H wag(–CH2X), C–O stretch & aromatic amines, alkyl halides, alcohols, carboxylic acids, esters, ethers, aromatic amines & \\
9 & 1377.33 & C–H bend & alkyl halides, alcohols, carboxylic acids, esters, ethers, aromatic amines & \\
10 & 1409.55 & C–C stretch (in–ring) & Aromatics & \\
11 & 1463.71 & C–H bend, C–C stretch (in–ring) & Alkanes, aromatics & \\
12 & 1604.70 & N–H bend & 1º amines & \\
13 & 1650.62 & N–H bend, = C = C– stretch & 1º amines, alkenes & \\
14 & 1709.51 & C = O stretch & α, β-unsaturated aldehydes, ketones & Alkanes, carboxylic acids \\
15 & 2852.41 & C–H stretch, O–H stretch & Alkanes, carboxylic acids & \\
16 & 2922.15 & C–H stretch, O–H stretch & Alkanes, carboxylic acids & \\
17 & 2957.39 & C–H stretch, O–H stretch & Alkanes, carboxylic acids & \\
18 & 3010.03 & =C–H stretch, C–H stretch & Alkanes, aromatics & \\
19 & 3331.81 & N–H stretch, O–H stretch (H–bonded) & 1º, 2º amines, amides, alcohols, phenols & \\
\hline
\end{tabular}
\end{table}
Phytochemical composition of MEJR

The methanolic extract of *J. regia* male flower (MEJR) were selected for phytochemical analysis by using HR-LCMS, on the basis of significant antioxidant, extraction yield (%), TPC and TFC content, and antimicrobial activity. With the retention times, absorbance spectra and MS data, chemical composition of MEJR possess 26 bioactive compounds Fig. 2. The chemical formulae, retention time, and mass of the compounds are listed in Table 4. Some of the above compounds have already been identified in different parts of *J. regia* L. from the different ecological conditions like Arginine in nuts of *J. regia* from New Zealand,[38] Docosahexyanoic acid in nuts from six *J. regia* Cultivars grown in Portugal,[48] 11-amino-undecanoic acid (ursolic acid) were reported in green husks of *J. regia* in China,[49] 2,2-Dimethyl-3-Oxo-Butyric Acid 2-Trimethylsilyl; Quercetin-3-O-glucuronide; Hexanoic acid, trimethylsilyl ester; Oleic

![Figure 1. FTIR spectrum of MEJR.](image1)

![Figure 2. HR-LCMS spectrum of MEJR.](image2)
Acid; n-Hexadecanoic acid and 1,2-Benzene dicarboxylic acid, bis-(2-methyl propyl) ester were reported in leaves, green husk, stem bark, and nuts of *J. regia*.\[50,51,10,52\] Chemical constituents like, Elephantopin, Gamma-L-Glutamyl-cysteine, Artemisinin, Madecassic acid, Dihydromyricetin, Swietenine, Securinine, and Sphinganine were reported first time from the *J. regia* as per our best knowledge. The differences in the phytochemistry may be varying by season, habitat, or the ecological conditions of plants.

**Conclusion**

The results obtained from the study showed that male flower of *J. regia* possess significant antioxidant and antimicrobial activity and can be used as an easily accessible source of natural

| Rt (min) | Mass (m/z) | Identification | Molecular formula | Molecular weight (g/mol) |
|---------|------------|----------------|-------------------|--------------------------|
| 4.98    | 126.0156   | Unknown        | C6 H14 N4         | 174.20                   |
| 5.89    | 174.1094   | Arginine       | C22 H32 O2         | 328.496                  |
| 7.149   | 316.1474   | 4,7,10,13,16,19-Docosahexaynoic acid (omega-3 fatty acid) | C19 H20 O7     | 360.362                  |
| 7.977   | 360.1231   | Elephantopin   | C8 H14 N2 O5 S     | 250.269                  |
| 8.399   | 250.0636   | gamma-L-Glutamyl-Lcysteine | C15 H22 O5     | 282.332                  |
| 9.79    | 282.142    | Artemisinin    | C11 H23 N O2       | 201.31                   |
| 9.795   | 201.1706   | 11-amino-undecanoic acid (ursolic acid)  | C11 H20 N2 O3   | 228.292                  |
| 10.046  | 228.1448   | Pro-Ile        | C10 H19 N3 O4      | 245.279                  |
| 10.817  | 138.0671   | Unknown        | C9 H17 N O5        | 219.24                   |
| 11.26   | 173.1058   | Unknown        | C7 H6 O5           | 170.12                   |
| 11.522  | 185.1419   | Unknown        | C10 H11 N O        | 161.20                   |
| 11.839  | 245.1352   | Gly-Ala-Val    | C12 H20 O3         | 261.32                   |
| 12.475  | 219.1084   | Pantothenic Acid (vitamin B5)  | C15 H12 O8      | 320.25                   |
| 13.096  | 136.0134   | 3,4,5-trihydroxy benzoic acid (Gallic acid) | C18 H32 O2     | 280.45                   |
| 13.608  | 161.083    | Indole-3-ethanol | C13 H15 N O2       | 217.268                  |
| 14      | 610.0622   | Quercetin-3-O-rutinoside | C10 H20 N2 O3   | 228.292                  |
| 14.911  | 217.1105   | Securinine     | C10 H19 N3 O4      | 245.279                  |
| 14.686  | 172.1164   | Decanoic acid  | C10 H20 N2 O3      | 228.292                  |
| 15.024  | 240.195    | Unknown        | C15 H12 O8         | 320.25                   |
| 15.354  | 504.3487   | Madecassic Acid | C18 H32 O2     | 280.45                   |
| 15.854  | 320.0514   | Dihydromyricetin | C13 H15 N O2       | 217.268                  |
| 16.411  | 280.342    | (9Z,12Z)-9,12-Octadecadienoic acid | C15 H12 O8      | 320.25                   |
| 16.601  | 320.0512   | Dihydromyricetin (Ampelopsin) | C16 H35 N O2  | 273.469                  |
| 18.012  | 273.2655   | C16 Sphinganine | C32 H40 O9        | 568.661                  |
| 18.765  | 315.2768   | Unknown        | C14 H28 O2         | 256.43                   |
| 19.041  | 478.12     | Quercetin-3-O-glucuronide (Miquelianin) | C16 H22 O4     | 278.3435                  |
| 19.227  | 460.2321   | Gln-Trp-Lys    | C18 H34 O2         | 282.47                   |
| 19.667  | 282.2231   | (9Z)-Octadecenoic acid (Oleic acid) | C32 H40 O9      | 568.661                  |
| 20.507  | 304.2999   | Unknown        | C14 H28 O2         | 256.43                   |
| 20.789  | 363.3137   | Unknown        | C16 H22 O4         | 278.3435                  |
| 21.354  | 568.273    | Swietenine     | C18 H28 O2         | 276.40                   |
| 21.976  | 256.321    | n-Hexadecanoic acid (Palmitic acid) | C32 H40 O9      | 568.661                  |
| 22.035  | 278.235    | Benzene dicarboxylic acid, bis (2-methyl propyl) ester | C14 H28 O2     | 256.43                   |
| 22.593  | 276.1869   | 4-oxo-9Z,11Z,13E,15E-octa-decatetraenoic acid (unsaturated fatty acids in plants) | C18 H28 O2  | 276.40                   |
| 24.89   | 393.3705   | Unknown        | C18 H28 O2         | 276.40                   |

Rt: Retention time
bioactive compounds. However, tested strains were more susceptible to MEJR as compared to EEJR and AEJR and significant free radical scavenging activity was also observed in MEJR. Seven new bioactive compounds were also identified from the MEJR along with other known compounds. Thus, the study explored the fact that *J. regia* L. have great reservoir of new antioxidant and antimicrobial agents and hence demonstrated that *J. regia* L. is a potential source of bioactive compounds can be used as functional food supplements to deter the need of antioxidants as well as microbes related to digestive and gastrointestinal tract, which are further needed to be explored in more details. Therefore, further studies are in progress to isolate the antioxidant and antimicrobial agents from MEJR using different spectroscopic techniques.

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