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Research Article

Comparative Phytochemical Evaluation and Biological Activities of Adi Pepper (A Farmer's Variety) with *P. nigrum*

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ABSTRACT

The "King of Spices," *Piper nigrum*, is one of 75 spice varieties cultivated in India. Adi pepper, a farmer's variety registered with the Protection of Plant Varieties and Farmers' Rights (PPVFR) Authority, was compared with *P. nigrum* based on its phytochemical, antioxidant, and antibacterial activities. Despite comparable phytochemical screening, Adi pepper ethanolic extract had greater total phenolic and flavonoid content (30.66 ± 0.13 mg GAE/g DCE and 43.08 ± 0.04 mg QE/g DCE). In antioxidant analyses, Adi pepper ethanolic extract exhibited a lower IC_{50} value in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (IC_{50} = 85.14 ± 0.12) compared with *P. nigrum* (IC_{50} = 94 ± 0.12). Reducing ability was also higher in Adi pepper ethanolic extract (0.431 ± 0.00) compared to *P. nigrum* (0.254 ± 0.00) in the reducing power assay. Adi pepper ethanol extract also presented a greater inhibition zone against *S. typhi* (18 ± 0.00 mm) and lower minimum inhibitory concentration (MIC) values (0.625 mg/mL) among the tested pathogens. Gas chromatography-mass spectrometry (GC-MS) analysis identified antimicrobial, anti-inflammatory, and insecticidal alkaloids as well as amide-type alkaloids in both types. Both peppers, in general, showed antioxidant and antibacterial activity, but Adi pepper slightly more, perhaps due to specific phytoconstituents. Further investigation of such bioactive components could enhance their culinary, medicinal, and other applications.

Introduction

The Piperaceae family comprises approximately 3,600 species of considerable culinary and medicinal importance, particularly *Piper betle*, *P. longum*, and *P. nigrum* (black pepper).[1,2] Over 100 species of *Piper* may be found in India, primarily in the Western Ghats of South India and the northeastern Himalaya.[3] *P. nigrum,* the "King of Spices," renowned for its pungent fruits, flourishes in tropical humid environments, depending on rainfall, temperature, and soil conditions.[4,5]

P. nigrum is known to have a variety of pharmacological activities, including anti-inflammatory, anticancer, hepatoprotective, immunomodulatory, analgesic, and antipyretic effects, amongst others.[6] Also, solvent extracts of *P. nigrum* exhibit a high level of DPPH scavenging activity, which is comparable to that of commercial antioxidants.[7,8] There is strong antimicrobial and antifungal action in many of the *P. nigrum* extracts as well.[9-11] Additionally, *P. nigrum* contains phytochemical contents in the form of alkaloids, terpenoids, and flavonoids, the most important among which is piperine- an alkaloid, having the highest bioactivity, showing anticancer, antibacterial, and anti-inflammatory effects.[12]

The conservation of biodiversity is significantly influenced by native and indigenous crops, which are naturally adapted to the local environment and necessitate minimal maintenance. An example of one such crop is Adi pepper, which is endemic to the Kodagu district of Karnataka. Due to its drought-tolerant, low-maintenance, and disease-resistant qualities, Adi pepper thrives in high-altitude, wet, and foggy conditions.

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Farmers' varieties, including the recently described "Adi pepper," remain unexplored, despite the extensive research conducted on *P. nigrum* and its cultivars (Fig.1). A spice and potential source of bioactive compounds, "Adi pepper" is cultivated in Karnataka, India, and is registered with the Protection of Plant Varieties and Farmers' Rights (PPVFR) Authority. Mr. Poonacha N. N., a farmer from Garvale, Karnataka, registered this Adi pepper. Contrary to the extensively researched and widely cultivated "*P. nigrum*," the farmer's variety "Adi pepper" provides a distinctive opportunity to assess its potential.

The taxonomic status of Adi pepper, despite its identification as *P. relictum*, is still unclear, underscoring the importance of the study. The necessity for a more thorough comprehension is underscored by the fact that both *P. nigrum* and Adi pepper are often called black pepper. Comparative phytochemical analysis and biological characteristics of Adi pepper with *P. nigrum* were conducted in order to fill in these voids and possibly help clarify their potential uses. A thorough examination and understanding of these distinctions will enhance our knowledge of these spices and their culinary and medicinal uses.

Materials And Methods

Collection of Plant Material

Seeds of the Adi pepper and *P. nigrum* were procured directly from farmers in the Garvale region, Kodagu. Subsequently, the following evaluation methodologies were implemented to conduct comparative analyses of the collected materials.

Preparation of the Solvent Extract using the Maceration Technique

Seed samples were uniformly powdered using a Waring blender, and oleoresins were extracted by maceration with solvents (petroleum ether, chloroform, ethanol, and methanol) at a 1:5 (w/v) solid-to-solvent ratio. The seed extracts were concentrated, placed in labelled pre-weighed tubes, and utilized for subsequent analysis [13].

Qualitative Analysis

Preliminary qualitative phytochemical analysis

Preliminary phytochemical screening of the seed extracts was conducted using established protocols reported in earlier studies.[14-18] Each extract (1g) was dissolved in its respective solvent and tested for the presence of various phytochemicals. The tests included Wagner's test, Dragendorff's test, and Hager's test for alkaloids; Keller-Killiani's test, the sulfuric acid test, and Modified Borntrager's test for glycosides; Benedict's test and Fehling's test for carbohydrates; the gelatin test and Braymer's test for tannins; the alkaline reagent test and ferric chloride test for flavonoids; the ferric chloride test for phenols; Libermann-Burchard's test for steroids; Salkowski's test for terpenoids; and the foam test for saponins.

a) b)

Fig. 1: Seeds of a) Adi pepper and b) *P. nigrum*

Quantitative Phytochemical Analysis

Estimation of total phenolic content (TPC)

To determine phenolic content in the seed extracts, a colorimetric procedure involving the Folin–Ciocalteu reagent was followed.[19] In brief, 0.5 mL of the seed extract (1-mg/mL) or a gallic acid standard solution was introduced into a test tube, followed by 5 mL of diluted Folin–Ciocalteu reagent (1:10 v/v). After thorough mixing, 4 mL of 1 M sodium carbonate was added. The reaction mixture was kept at ambient temperature for 15 minutes before its absorbance was recorded at 765 nm. A gallic acid calibration curve (20–100 µg/mL) was prepared for quantification. Phenolic content was calculated and presented as milligrams (mg) of gallic acid equivalents (GAE) per gram (g) of dry crude extracts (DCE). Each sample was analysed in triplicate to ensure accuracy.

Estimation of flavonoid content

Flavonoid estimation in the seed extracts was performed through an aluminium chloride-based spectrophotometric technique, with reference to earlier methods [20]. Briefly, 1 mL of the seed extracts (1-mg/mL) or quercetin standard was reacted with 1-mL of 2% aluminium chloride prepared in methanol. The mixture was allowed to incubate at room temperature for 15 minutes, facilitating the development of a yellow flavonoid–aluminium complex. Absorbance was subsequently read at 430 nm using a UV–visible spectrophotometer. Quantification was achieved using a quercetin calibration curve (20–100 µg/mL), and results were reported as milligrams (mg) of quercetin equivalents (QE) per gram (g) of dry crude extract (DCE).[21,22] Each sample was tested in triplicate, and the average readings were considered for analysis.

Antioxidant Assay

DPPH radical scavenging activity

The free radical scavenging activity was quantified using the DPPH technique, which was developed by Blois[23] with minor modifications. Seed extracts (20–100 µg/mL) were combined with a 0.1 mM methanolic DPPH solution in a 3:1 ratio. The absorbance was recorded at 517 nm after the

incubation period of 30 minutes. Butylated hydroxytoluene (BHT) was employed as the standard. The percentage of radical scavenging activity, which indicates hydrogen-donating ability, was calculated using the formula

$$\% \text{ inhibition} = (A_o - A_t) / A_o \times 100,$$

where A_o is the absorbance of the control (blank) and A_t is the absorbance of the seed extract. The IC_{50} values for standard and seed extracts were calculated from the graph of percentage inhibition vs. concentration.[24]

Reducing power assay

The ability of the extract to reduce Fe^{3+} to Fe^{2+} was assessed using a modified method initially developed by Oyaizu[25] and later refined by Fejes *et al.*[26]. The absorbance corresponding to the formation of Perl's Prussian blue complex was measured at 700 nm. To 1 mL of the seed extracts or standard solution (100–500 µg/mL), 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution were added and incubated at 50°C for 30 minutes. After adding 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant (2.5 mL) was then combined with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride, and absorbance was measured at 700 nm. Butylated hydroxytoluene (BHT) was used as the standard, and phosphate buffer (pH 6.6) served as the blank. The intensity of absorbance was directly proportional to the reducing potential of the sample.[27]

Antibacterial Assay

Well-diffusion method

The antimicrobial activity was assessed using the well diffusion method [28] against selected gram-positive bacteria (*Bacillus subtilis* [MTCC-121T], *Staphylococcus aureus* [MTCC-740]) and gram-negative bacteria *(Escherichia coli* [MTCC-7410], *Salmonella typhi* [MTCC-733]). Nutrient agar plates were uniformly swabbed with 50 µL of a bacterial inoculum. Each well, prepared using a sterile cork borer, was loaded with 100 µL of the seed extracts (100 mg/mL), dimethyl sulfoxide (DMSO) as the negative control, or streptomycin (1-mg/mL) as the positive control. The inoculated plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones was measured in millimeters[29].

Determination of minimum inhibitory concentration (MIC) of antibacterial active extracts

Minimum inhibitory concentration (MIC) of antibacterial extracts was obtained using sterile 96-well microplates. Initially, each well was loaded with 100 µL of Muller-Hinton broth. In the first well, 100 µL of a 10 mg/mL seed extract solution was dispensed. The extract was next serially diluted to provide a range of 0.5 to 0.039 mg/mL. In the same manner, reference antibiotic (streptomycin) was achieved with a concentration of 0.05 to 0.00039 mg/mL. Each well was inoculated with 20 µL of the bacterial suspension. The initial two wells were sterility (broth only) and negative (broth + inoculum) controls. After sealing, the plates were incubated at 37°C for a period of 24 hours. To assess microbial viability, 20 µL of a 2 mg/mL solution of 2,3,5-triphenyltetrazolium chloride (TTC) was added to each well as a growth indicator. MIC was determined as the lowest concentration of the extract at which no colour change was observed, indicating complete inhibition of microbial growth [30–32].

GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of ethanolic extracts of each pepper was done employing a Shimadzu Nexis GC-2030 system equipped with an AOC-30/20i auto sampler. Using an SH-I-5- 5Sil MS capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm thickness). Mass spectra of compounds found using GC-MS Solutions software were matched with data from the NIST 11 and Wiley8 spectral libraries.

Statistical Analysis

The analysis of the triplicate results was conducted using ANOVA in SPSS software Inc. 16.0. Effects that were statistically significant were shown by F values ($p \leq 0.05$) in Tukey's HSD test means.

Results And Discussion

Qualitative Analysis

Preliminary qualitative phytochemical analysis

Phytochemical screening of solvent extracts from Adi pepper and *P. nigrum* revealed alkaloids in all solvents (petroleum ether, chloroform, ethanol, and methanol) (Table 1). Both plants showed general glycosides (sulfuric acid test) and cardiac glycosides (Keller-Killiani's test) in petroleum ether, chloroform, and ethanol extracts. Anthraquinone glycosides were detected in chloroform extracts, as indicated by the Modified Borntrager's test. Analysis of carbohydrates indicated that chloroform extracts tested negative in Benedict's test but positive in Fehling's test. Terpenoids were detected in all extracts. Ethanol and methanol extracts contained tannins, flavonoids, and phenols but no steroids. A comparison between Adi pepper and *P. nigrum* revealed no major differences. Prior investigations demonstrated that the extraction of alkaloids, phenols, tannins, and flavonoids from *P. nigrum* varied depending on the solvent used, indicating the solvent's influence on phytochemical yield. [33] These bioactive compounds likely contributed to the significant biological activity observed in both spices.

Table 1: Phytochemical analysis of solvent extracts of Adi pepper and *P. nigrum*

Tests	*Petroleum ether*		*Chloroform*		*Ethanol*		*Methanol*	
	Adi pepper	*P. nigrum*	*Adi pepper*	*P. nigrum*	*Adi pepper*	*P. nigrum*	*Adi pepper*	*P. nigrum*
1. Test for alkaloids								
a) Wagner's test	+	+	+	+	+	+	+	+
b) Dragendorff's test	+	+	+	+	+	+	+	+
c) Hager's test	+	+	+	+	+	+	+	+
2. Test for glycosides								
a) Keller-Killiani's test	+	+	+	+	+	+	-	-
b) Sulfuric acid test	+	+	+	+	+	+	-	-
c) Modified Borntrager's test	-	-	+	+	-	-	-	-
3. Test for carbohydrates								
a) Benedict's test	-	-	-	-	-	-	-	-
b) Fehling's test	-	-	+	+	-	-	-	-
4. Test for tannin								
a) Gelatin test	-	-	-	-	+	+	+	+
b) Braymer's test	-	-	-	-	+	+	+	+
5. Test for flavonoids								
a) Alkaline reagent test	-	-	-	-	+	+	+	+
b) Ferric chloride test	-	-	-	-	+	+	+	+
6. Test for phenols - Ferric chloride test	-	-	-	-	+	+	+	+
7. Test for steroids - Libermann-Burchard's test	-	-	-	-	-	-	-	-
8. Test for terpenoids - Salkowski's test	+	+	+	+	+	+	+	+
9. Test for saponin - Foam test	-	-	-	-	-	-	-	-

Note: "+": Present; "-": Absent

Table 2: Total phenolic and flavonoid contents in the solvent extracts of Adi pepper and *P. nigrum*

Extracts	*Petroleum ether*		*Chloroform*		*Ethanol*		*Methanol*	
	Adi pepper	*P. nigrum*	*Adi pepper*	*P. nigrum*	*Adi pepper*	*P. nigrum*	*Adi pepper*	*P. nigrum*
Total phenolic content (TPC) (mg GAE/g of DCE)	-	-	-	-	30.66 ± 0.13	29.18 ± 0.13	21.58 ± 0.09	18.21 ± 0.17
Flavanoid content (mg QE/g of DCE)	-	-	-	-	43.08 ± 0.04	38.38 ± 0.04	32.65 ± 0.01	30.63 ± .028

Each values represent the mean ± SEM; (n=3); Note: "-" Not determined

Quantitative Phytochemical Analysis

Estimation of total phenolic content (TPC)

Ethanol extracts of Adi pepper (30.66 ± 0.13 mg GAE/g of DCE) and *P. nigrum* (29.18 ± 0.13 mg GAE/g of DCE) had the highest TPC value among pepper extracts, followed by methanol extracts (Table 2). Earlier studies on *P. nigrum* extracts presented variable total phenolic content (TPC): 45.08 µg gallic acid/g *P. nigrum* for ethanolic extract,[34] 1.728 mg/g of gallic acid equivalent for methanolic extract,[33] and 120 ± 3.5 mg GAE/g for hydroalcoholic extract.[35] As the solvent and extraction procedure of *P. nigrum* extract varied in the study, TPC also varied.

Estimation of flavonoid content

The highest amount of flavonoids was found in the ethanol extracts of Adi pepper (43.08 ± 0.04 mg QE/g of DCE) and *P. nigrum* (38.38 ± 0.04 mg QE/g of DCE), followed by the methanol extracts (Table 2). Previous studies of *P. nigrum* extracts have also shown high variations in flavonoid contents of 1.087 ± 0.002 µg/g quercetin equivalent in methanolic extract[33] to 75 ± 2.2 mg QE/g in

hydroalcoholic extract[35] based on solvent and extraction conditions.

Antioxidant Assay

DPPH radical scavenging activity

The DPPH radical scavenging effect of the standard (BHT) and pepper seed extracts was concentration-dependent (20–100 µg/mL). The ethanolic extract of Adi pepper possessed the maximum activity (57.45 ± 0.13%), whereas *P. nigrum* ethanolic extract displayed 51.23 ± 0.23%. Furthermore, lower IC_{50} values suggest greater DPPH radical scavenging activity. The ethanol extract contained the lowest IC_{50} values for Adi pepper (IC_{50} = 85.14 ± 0.12) compared with *P. nigrum* (IC_{50} = 94 ± 0.12), next being methanol, petroleum ether, and chloroform extracts (Fig. 2a). *P. nigrum* extracts have shown concentration- and solvent-dependent antioxidant activity in previous studies. The earlier reports on ethanolic extracts showed 48% scavenging activity at 75 µg/mL, though not as potent as reference antioxidants, including BHA, tocopherol, and BHT.[36] The ethanolic extract showed the highest antioxidant activity (65.59% at 50 µg/mL), comparable to BHT (60%), while the aqueous extract exhibited the lowest scavenging effect.[34] In addition, DPPH radical scavenging activity was concentration-dependent, with an EC_{50} of 0.152 ± 0.001 mg/mL for ethanolic extracts [37].

Reducing power assay

The reducing power activity of the standard (BHT) and pepper seed extracts increased with concentration (100–500 µg/mL). The maximum absorbance (0.431 ± 0.00) was shown by the ethanol extract of Adi pepper among the other extracts. Similarly, the ethanol extract of *P. nigrum* demonstrated the highest absorbance (0.254 ± 0.00). For both Adi pepper and *P. nigrum*, the ethanol extract exhibited the greatest reducing power, followed by methanol, petroleum ether, and chloroform extracts (Fig. 2b). In the previous study, the ethanolic extract of *P. nigrum* exhibited a reducing power of 0.8559 ± 0.070. [36] Another research indicated that activity increased progressively with extract concentrations of 25 to 75 µg/mL [34]. A concentration-dependent increase in reducing power was observed, largely attributed to phenolic and flavonoid constituents, underscoring their vital role in the antioxidant potential of *P. nigrum*.[37]

Antibacterial Assay

Well-diffusion method

The antibacterial activity of Adi pepper and *P. nigrum* was assessed using the well diffusion assay with various solvent extracts (Table 3). All tested extracts were inactive against *S. aureus*, showing no measurable inhibition. Maximum antibacterial activity was observed with the ethanolic extract of Adi pepper against *S. typhi*, *B. subtilis*, and *E. coli*, with inhibition zones of 18.00 ± 0.00, 16.33 ± 0.66, and 16.33 ± 0.66 mm, respectively. The chloroform extract of *P. nigrum* demonstrated the highest inhibition zones against *B. subtilis* and *E. coli* (14.33 ± 0.33 mm), whereas *S. typhi* was most susceptible to the ethanolic extract (14.33 ± 0.33 mm). The ethanolic extracts of Adi pepper and *P. nigrum* differed significantly in their ability to inhibit *S. typhi* (Adi pepper: 18.00 ± 0.00 mm; *P. nigrum*:14.33 ± 0.33 mm), *B. subtilis* (Adi pepper: 16.33 ± 0.66 mm; *P. nigrum*: 13.33 ± 0.66 mm), and *E. coli* (Adi pepper: 16.33 ± 0.66 mm; *P. nigrum*: 13.66 ± 0.66 mm). Notable variations were also evident in the activity of chloroform extracts against *S. typhi* (Adi pepper: 15.66 ± 0.33 mm; *P. nigrum*: 13.33 ± 0.66 mm). Streptomycin inhibited all pathogens, while DMSO showed no activity (Fig. 4). Previous research has shown *P. nigrum* extracts

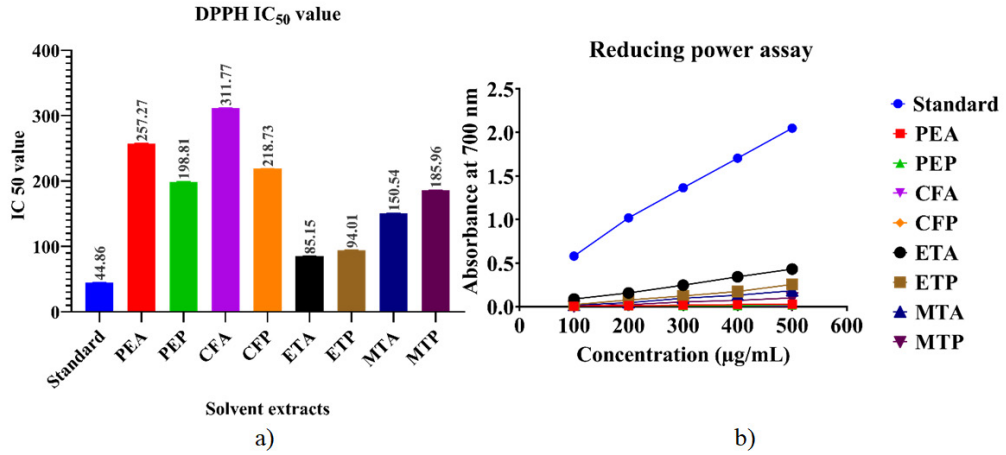

Fig. 2: a)Comparative IC_{50} value of solvent extracts of Adi pepper with *P. nigrum* by DPPH method. b)Comparative antioxidant activity of solvent extracts of Adi pepper with *P. nigrum* by reducing power assay. Note: PEA- Petroleum ether Adi pepper; PEP- Petroleum ether *P. nigrum*; CFA- Chloroform Adi pepper; CFP- Chloroform *P. nigrum*; ETA- Ethanol Adi pepper; ETP- Ethanol *P. nigrum*; MTA- Methanol Adi pepper; MTP- Methanol *P. nigrum*

have wide-spectrum antibacterial activity, with ethanolic and chloroform extracts inhibiting gram-positive as well as gram-negative bacteria like *B. subtilis*, *S. aureus*, *E. coli*, *S. typhi*, and *Proteus* sp.[34, 38] Greatest inhibition occurred in ethanolic extracts with 20.1 ± 0.72 mm zones for *B. subtilis*, 16.1 ± 0.58 mm for *E. coli*, and 15.6 ± 1.2 mm for *S. aureus*.[13]. Hydroalcoholic extracts were also highly active, owing to the presence of piperine.[35] The findings demonstrate the role of solvent type and bacterial strain in the antimicrobial activity of *P. nigrum.*

Determination of minimum inhibitory concentration (MIC) of antibacterial active extracts

Seed extracts exhibited MICs ranging from 1.250 to 0.625 mg/mL. Among them, Adi pepper ethanolic extract was most potent, with an MIC of 0.625 mg/mL against *E. coli*, *S. typhi*, and *B. subtilis*. The chloroform extract of Adi pepper also showed a low MIC (0.625 mg/mL) against *E. coli*. In *P. nigrum*, only the chloroform extract recorded the lowest MIC (0.625 mg/mL) against *E. coli*. Significant variations in MIC were observed between the ethanolic extracts of Adi pepper (0.625 mg/mL) and *P. nigrum* (1.250 mg/mL) across all pathogens. Additionally, chloroform extracts of Adi pepper (0.625 mg/mL) and *P. nigrum* (1.250 mg/mL) exhibited notable differences in activity against *S. typhi* (Table 4). In the earlier study, MIC values of 156.25 µg/mL against *S. aureus* and *B. subtilis* and 1250 µg/mL against *E. coli* and *K. pneumoniae* were reported in ethanol extracts of *P. nigrum*.[34] Lower MIC values were obtained against *S. aureus* and *E. coli* (31.25 µg/mL) and against *B. subtilis* (3.9 µg/mL).[13] Hydroalcoholic extracts also showed high activity with MICs varying from 3.5–4.2 mg/mL.[35] These differences are probably a result of different solvent systems, efficiencies of extraction, and concentrations of active constituents.

GC-MS Analysis

The GC-MS analysis of the ethanolic extracts from Adi pepper (Table 5) and *P. nigrum* (Table 6) identified alkaloids and amide-type alkaloids, common in *Piper* species, with antibacterial, anti-inflammatory, and insecticidal properties. In Adi pepper, 25 compounds were detected, with piperine (47.12%) as the main alkaloid, followed by piperidine (10.37%), piperolein B (7.46%), and pipersintenamide (4.99%). Minor compounds included piperundecalidine (2.64%) and pipericine (2.18%).

Table 3: Antibacterial activity of solvent extracts of Adi pepper and *P. nigrum* by well diffusion assay

Solvents	*Petroleum ether*		*Chloroform*		*Ethanol*		*Methanol*		*Positive control*	*Negative control*
Pathogen	*Adi pepper*	*P. nigrum*	*Adi pepper*	*P. nigrum*	*Adi pepper*	*P. nigrum*	*Adi pepper*	*P. nigrum*	*Standard-Streptomycin*	*DMSO*
S. aureus	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	0 ± 0.00^{b}	30.00 ± 0.00^{a}	0 ± 0.00^{b}
B. subtilis	14.00 ± 0.00^{de}	12.66 ± 0.33^{e}	16.00 ± 0.00^{bc}	14.33 ± 0.33^{cde}	16.33 ± 0.66^{b}	13.33 ± 0.66^{de}	15.00 ± 0.00^{bcd}	14.00 ± 0.00^{ed}	28.33 ± 0.33^{a}	0 ± 0.00^{f}
E. coli	13.66 ± 0.33^{d}	13.66 ± 0.33^{d}	15.66 ± 0.33^{bc}	14.33 ± 0.33^{cd}	16.33 ± 0.66^{b}	13.66 ± 0.66^{d}	15.00 ± 0.00^{bcd}	14.00 ± 0.00^{cd}	28.00 ± 0.00^{a}	0 ± 0.00^{e}
S. typhi	14.00 ± 0.00^{de}	13.00 ± 0.00^{e}	15.66 ± 0.33^{c}	13.33 ± 0.66^{de}	18.00 ± 0.00^{b}	14.33 ± 0.33^{cd}	14.00 ± 0.00^{de}	13.00 ± 0.00^{e}	30.66 ± 0.33^{a}	0 ± 0.00^{f}

The zone of inhibition was measured in millimeters (mm). Each values represent the mean ± SEM; (n = 3). Based on Tukey's b HSD, there is no significant difference ($p < 0.05$) between means that are followed by the same letter or letters within the same column.

Table 4: Minimum inhibitory concentration (MIC) values of Adi pepper and *P. nigrum* solvent extracts against tested bacteria

Name of the pathogen	*Standard -Streptomycin (mg/mL)*	*Petroleum ether*		*Chloroform*		*Ethanol*		*Methanol*	
		Adi pepper (mg/mL)	*P. nigrum (mg /mL)*	*Adi pepper (mg/mL)*	*P. nigrum (mg /mL)*	*Adi pepper (mg/mL)*	*P. nigrum (mg /mL)*	*Adi pepper (mg/mL)*	*P. nigrum (mg /mL)*
S. aureus	-	-	-	-	-	-	-	-	-
B. subtilis	0.0125	1.250	1.250	1.250	1.250	0.625	1.250	1.250	1.250
E. coli	0.00625	1.250	1.250	0.625	0.625	0.625	1.250	1.250	1.250
S. typhi	0.00312	1.250	1.250	0.625	1.250	0.625	1.250	1.250	1.250

Values presented were average of triplicate experiments. Note: "-": Not determined

Table 5: GC-MS analysis of ethanol extract of Adi pepper

Peak no.	*Retention time*	*Area %*	*Compound name*	*Mol. formula*
1	8.039	0.46	1,3-Dimethyl-1-cyclohexene	C_8H_{14}
2	8.088	0.36	1,3-Dimethyl-1-cyclohexene	C_8H_{14}
3	8.207	0.47	4-Terpineol	$C_{10}H_{18}O$
4	9.091	0.30	4(10)-Thujen-3-ol, acetate	$C_{12}H_{18}O_2$
5	9.229	0.30	Carvotanacetone	$C_{10}H_{16}O$
6	10.481	0.54	δ-Elemene	$C_{15}H_{24}$
7	11.078	0.64	Copaene	$C_{15}H_{24}$
8	11.353	1.67	β-Elemene	$C_{15}H_{24}$
9	11.353	0.72	3-Methyl-2-(3-methylpentyl)-3-buten-1-ol	$C_6H_{10}O$
10	11.703	8.63	Caryophyllene	$C_{15}H_{24}$
11	12.171	0.65	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	$C_{15}H_{24}$
12	12.488	1.21	Germacrene D	$C_{15}H_{24}$
13	12.605	1.00	β-Eudesmene	$C_{15}H_{24}$
14	13.280	2.29	Elemol	$C_{15}H_{26}O$
15	13.776	0.93	Caryophyllene oxide	$C_{15}H_{24}O$
16	14.640	3.28	Asarone	$C_{12}H_{16}O_3$
17	15.806	0.36	4-Methyldibenzothiophene	$C_{13}H_{10}S$
18	31.691	5.05	Piperidine	$C_{19}H_{21}NO_3$
19	32.187	5.32	Piperidine	$C_{19}H_{21}NO_3$
20	33.237	1.43	Piperidine, 1-(1-oxo-2-hexadecenyl)-, (E)-	$C_{21}H_{39}NO$
21	34.580	2.18	Pipericine	$C_{22}H_{41}NO$
22	36.497	47.12	Piperine	$C_{17}H_{19}NO_3$
23	37.083	2.64	Piperundecalidine	$C_{23}H_{29}NO_3$
24	37.285	4.99	Pipersintenamide	$C_{19}H_{23}NO_3$
25	40.627	7.46	Piperolein B	$C_{21}H_{29}NO_3$
		100%		

Bioactive substances like caryophyllene (8.63%), asarone (3.28%), and elemol (2.29%) were also present, likely contributing to the antibacterial activity (Fig. 4).

In *P. nigrum*, 20 compounds were identified, with piperine (53.08%) as the dominant component. Other significant compounds included N-Isobutyloctadeca-2,4,12-trienamide (7.05%), N-Isobutylicosa-2,4,14-trienamide (6.73%), and piperolein B (4.19%) *P. nigrum* against. Additional bioactive compounds included β-cis-caryophyllene (6.07%), β-bisabolene (4.79%) and copaene (2.00%) (Fig. 5). A previous report on ethanolic extracts of *P. nigrum* contained a mixture of terpenoids such as α-pinene, sabinene, β-myrcene, δ-3-carene, dl-limonene, α-phellandrene, α-humulene, trans-β-caryophyllene, α-copaene, and (-)-caryophyllene oxide.[13] In hexane extracts, the most abundant compound was piperine (14.22%), followed by piperanine, pellitorine, and diisocotyl phthalate,[39] which are believed to contribute to antibacterial activity. In spite of compositional differences, a common phytochemical fingerprint of fundamental

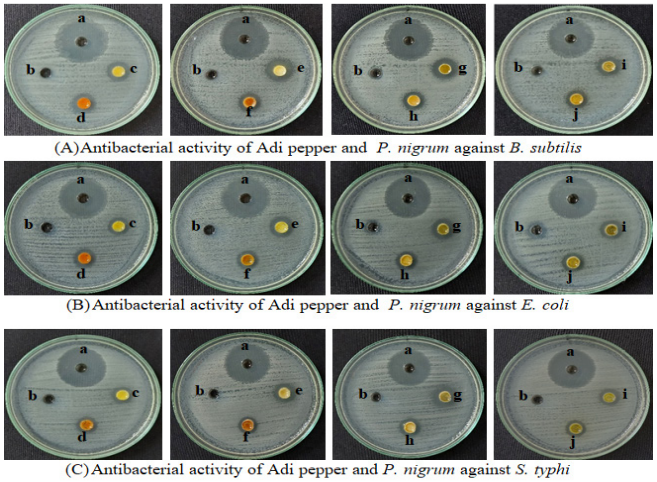

Fig. 3: Comparative antibacterial activity of solvent extracts of Adi pepper with *P. nigrum* against the tested pathogens by well diffusion assay. Note: a-Standard (Streptomycin); b- DMSO; c- Adi pepper petroleum ether; d- *P. nigrum* petroleum ether; e- Adi pepper chloroform; f- *P. nigrum* chloroform; g- Adi pepper ethanol; h- *P. nigrum* ethanol; i- Adi pepper methanol; j- *P. nigrum* methanol

Table 6: GC-MS analysis of ethanol extract of *P. nigrum*

Peak no.	*Retention time*	*Area %*	*Compound name*	*Mol. formula*
1	8.414	0.36	Dodecane	$C_{12}H_{26}$
2	11.074	2.00	Copaene	$C_{15}H_{24}$
3	11.269	0.40	Hexadecane	$C_{16}H_{34}$
4	11.698	6.07	β-cis-Caryophyllene	$C_{15}H_{24}$
5	12.735	4.78	beta.-Bisabolene	$C_{15}H_{24}$
6	12.909	1.18	(-)-β-Cadinene	$C_{15}H_{24}$
7	13.691	0.34	Ethyl dodecanoate	$C_{14}H_{28}O_2$
8	14.476	1.06	(-)-δ-Cadinol	$C_{15}H_{26}O$
9	18.454	1.97	Pellitorine	$C_{14}H_{25}NO$
10	31.678	1.18	Piperidine	$C_{19}H_{21}NO_3$
11	32.229	2.74	Piperanin	$C_{17}H_{21}NO_3$
12	32.570	1.31	Piperlonguminine	$C_{16}H_{19}NO_3$
13	34.231	3.72	Piperine	$C_{17}H_{19}NO_3$
14	34.465	7.05	(2E,4E,12E)-N-Isobutyloctadeca-2,4,12-trienamide	$C_{22}H_{39}NO$
15	34.574	2.52	(2E,4E)-N-Isobutyloctadeca-2,4-dienamide	$C_{22}H_{41}NO$
16	35.911	3.03	Piperylin	$C_{16}H_{17}NO_3$
17	36.491	49.36	Piperine	$C_{17}H_{19}NO_3$
18	37.411	3.25	(2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide	$C_{24}H_{43}NO$
19	37.607	3.48	(2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide	$C_{24}H_{43}NO$
20	40.616	4.19	Piperolein B	$C_{21}H_{29}NO_3$
		100%		

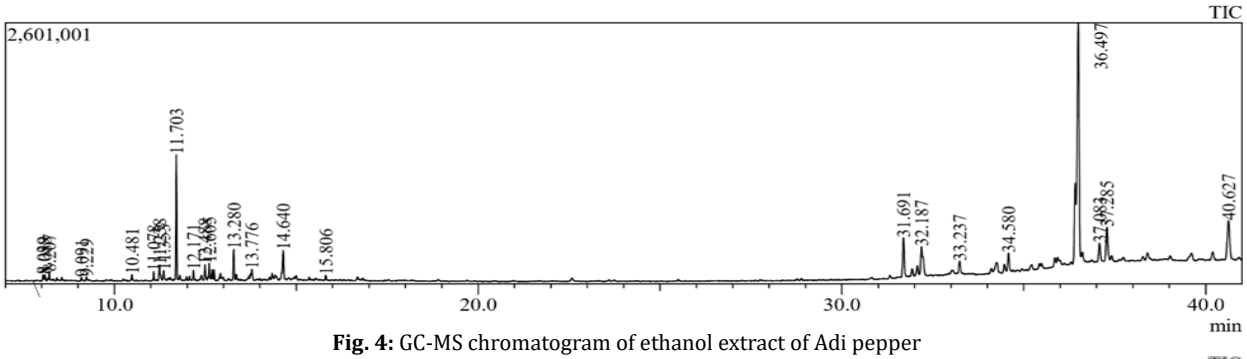

Fig. 4: GC-MS chromatogram of ethanol extract of Adi pepper

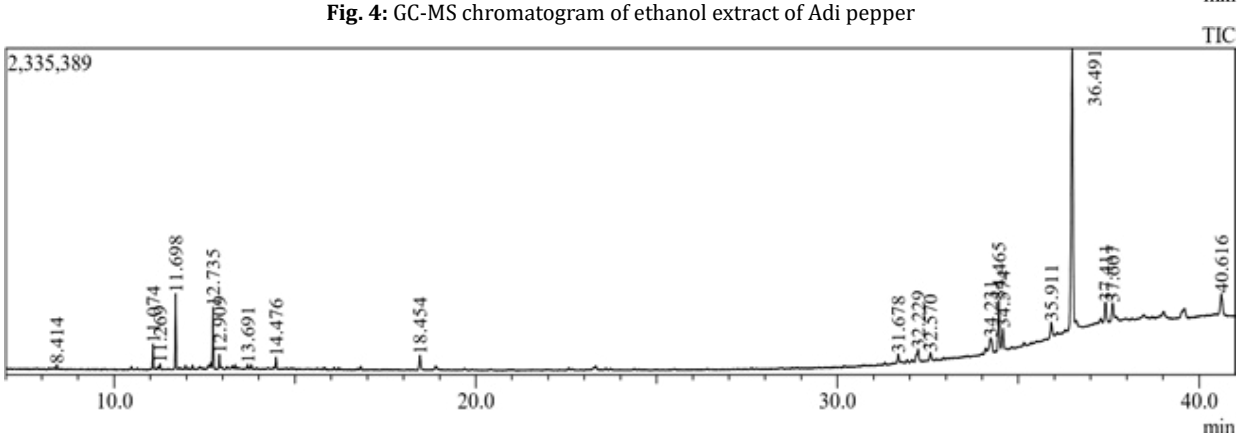

Fig. 5: GC-MS chromatogram of ethanol extract of *P. nigrum*

functional and therapeutic significance is indicated by the recurring presence of dominant compounds throughout the studies.

Conclusion

This study presents a comparative analysis of the phytochemical composition and biological activities of Adi pepper and *P. nigrum*. Although the phytochemical profiles of both peppers were basically comparable, the ethanolic extract of Adi pepper had somewhat greater total phenolic content, flavonoid levels, antioxidant capacity, and antimicrobial activity. These improved properties may be ascribed to distinctive bioactive constituents that are exclusive to Adi pepper. The findings highlight that Adi pepper, a locally cultivated farmer's variety, is as effective as *P. nigrum*, with slightly enhanced biological activity. These findings emphasize the need for more studies to identify and characterize specific bioactive components in Adi pepper. Such attempts could significantly increase its potential applications, including the food industry, medicine, and other industries.

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