

RESEARCH ARTICLE

Detection of Antioxidant and Antimicrobial Activity of Leaf Extract of *Jasminum azoricum*

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ABSTRACT:

Jasminum azoricum, which belongs to *Oleaceae* family¹, was chosen for this study since there is no detailed research done on this plant for its medicinal properties. The presence of various phytochemicals like tannins, alkaloids, steroids, polyphenol, terpenoids, glycosides, flavonoids, quinones and coumarines were examined using various extracts of *Jasminum azoricum*. The phytochemicals were further screened to determine the antioxidant and antimicrobial properties. The F5 fraction obtained from column chromatography of the acetone extract was examined by DPPH free radical scavenging method which revealed effective antioxidant activity when compared to other fractions. The F5 fraction of acetone extract of *J.azoricum* was further tested for antimicrobial activity which showed a reasonable anti-microbial activity for *B.cereus*, *S.aureus* and *Pseudomonas sp.*. Hence this leaf extract can be used for the production of novel bioactive substance which can induce antioxidant and antimicrobial defence in humans and can thereby serve to eliminate human ailments.

KEYWORDS: *Jasminum azoricum*, Phytochemicals, DPPH Assay, Anti-oxidant activity, Anti-microbial activity.

INTRODUCTION:

India, being one of the rich countries in terms of its flora, has about 18,000 flowering plant species out of which 2,500 species possess medicinal value. Jasmine, being traditionally used in the oil production was found to have medicinal properties². *Jasminum azoricum*, a woody perennial climber which bears green coloured pinnate leaves and sweet smelling flowers arranged in clusters. The medicinal properties of this plant are because of the presence of various phytochemicals. These phytochemicals may act as antioxidant^{3,4} and antimicrobial agent which can be used to treat heart disease, sunstroke, cancer and skin related ailments⁵. Antioxidants are compounds that inhibit oxidation of other molecules.

Oxidation is a chemical reaction which generates free radicals by transfer of electron or hydrogen. The free radicals start the chain reaction within cell leading to cell death⁶. Antioxidants on the other hand are reducing agents that remove the free radicals and prevent cell death. Example: thiol, polyphenol, ascorbic acid. The phytochemical compounds of *Jasminum azoricum* can be used as antioxidant. Virulent species are the ones that are of keen interest because they cause a variety of disease that are either transmissible or non-transmissible. Various chemical components of *J. azoricum* are used to control the growth of microorganisms and are termed as antimicrobials which include antibacterial and antifungal.

MATERIALS AND METHODS:

PREPARATION OF LEAF EXTRACT:

The leaves of *Jasminum azoricum* were collected from Avadi, Western part of Chennai (Tamil Nadu) and were authenticated. The collected leaves were shade dried for a week, powdered using clean mortar and pestle and then uniform powder is obtained by passing the sample through sieve. Uniformly powdered leaves were extracted with five different solvents namely ethanol,

water, chloroform, acetone and petroleum ether⁷. The extracts were prepared by mixing 2 grams of powdered leaves with about 25-30ml of the respective solvent. The extracts were then incubated overnight except for the aqueous extract (to control contamination). Aqueous extract was incubated in water bath maintained at 55°C for about 30 minutes. This results in separation of solvent layer containing the dissolved chemical constituents from the plant extract, which is then used for phytochemical analysis.

PHYTOCHEMICAL ANALYSIS:

The various phytochemical such as tannins, alkaloids, steroids, polyphenol, terpenoids, glycosides, flavonoids, quinones and coumarines present in the leaf extract of *J. azoricum* was estimated by the standard procedure^{8,9}.

QUALITATIVE ASSAY OF ANTI-OXIDANT ACTIVITY:

Anti-oxidant assay of *Jasminum azoricum* was estimated by using DPPH (1, 1 -Diphenyl-2-picrylhydrazyl) free radical scavenging method^{10,11,12}. 50µl of acetone leaf extract of *J. azoricum* was taken in a micro-titre plate and 100µl of 0.1% methanolic DPPH was added over the sample followed by incubation for 30 minutes in dark condition. The decolouration of DPPH from purple to yellow reveals the intensity of radical scavenging of the samples. The radical scavenging activity of the sample was calculated by the following formula¹³:

$$\text{Inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance of sample}) \times 100}{(\text{Absorbance of control})}$$

QUANTITATIVE ASSAY OF ANTI-OXIDANT ACTIVITY:

100µl of acetone leaf extract sample from qualitative assay were mixed with 2.7ml of Methanol followed by the addition of 200µl of 0.1% methanolic DPPH. The absorption maxima of the incubated suspension were measured using UV Double Beam Spectra Scan (Chemito, India) at 517nm for every 5 minutes interval.

The anti-oxidant activity of the sample was compared with known synthetic standard of 0.16% of Butylated Hydroxy Toluene (BHT). The residue of evaporated acetone extract was mixed with n-butanol and water (2:1), layers were allowed to separate and evaporated under vacuum. The extract is dissolved in methanol and filtered. The concentrated extract in methanol was separated and analyzed by column chromatography¹⁴.

ANTI- MICROBIAL ACTIVITY:

The acetone leaf extract of *Jasminum azoricum* plant was used for antibacterial study by the method proposed by Ozkan *et al.*, 2012¹⁵; Janarthanam and Sumathi, 2010¹⁶. Different concentrations (10mg, 20mg, and 30mg/ml) of the concentrated acetone leaf extract were tested for their antimicrobial activity against different pathogenic bacterial strains such as *Staphylococcus aureus*¹⁷, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas sp.* The bacterial cultures were grown in Muller Hinton Agar and Muller Hinton Broth (Himedia). Antibacterial activity was measured using the standard disc diffusion method on agar^{18,19}.

RESULTS AND DISCUSSION:

The leaf sample of *Jasminum azoricum* were extracted with five different solvents such as aqueous, ethanol, acetone, chloroform and petroleum ether and the phytochemical screening was done. The acetone extract revealed the presence of 8 phytochemicals such as tannins, flavonoids, quinones, cardio-glycosides, terpenoids, phenols, steroid and alkaloids (Table 1) out of 13 phytochemical tests. 3 phytochemicals such as tannins, quinones and steroid showed higher concentration in the acetone extract. Similarly the aqueous extract revealed the presence of 7 phytochemicals such as tannins, saponins, flavonoids, phenols, coumarins, betacyanins, steroids and alkaloid out of which saponins, alkaloids and betacyanins were found to be present in higher concentration. The presence of betacyanin, coumarin and saponin were found only in aqueous extract.

Table 1: Phytochemical screening results

Phytochemical	Aqueous	Ethanol	Acetone	Chloroform	Pet. Ether
Tannins	+	++	++	+	-
Saponins	++	-	-	-	-
Flavonoids	+	-	+	-	-
Quinones	-	-	++	-	-
Glycosides	-	-	-	-	-
Cardio glycosides	-	+	+	-	+
Terpenoids	-	+	+	+	+
Phenols	+	-	+	-	-
Coumarins	+	-	-	-	-
Steroids	+	++	++	+	-
Alkaloids	++	+	+	-	-
Anthocyanins	-	-	-	-	-
Betacyanins	++	-	-	-	-

Note: ++ (presence of phytochemical with high concentration), + (presence of phytochemical), - (absence of phytochemical)

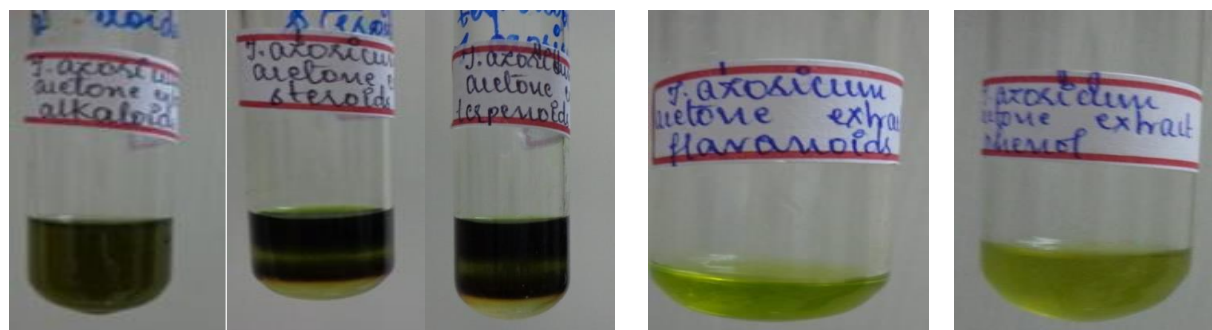


Fig.1: Photochemical Positive results of Acetone extracts

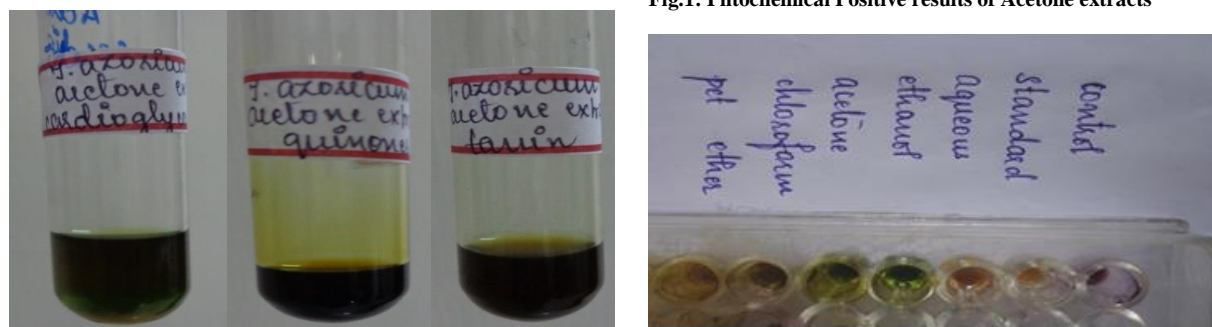


Fig.2: DPPH results of different extracts in a microtitre plate

Table 2: Quantitative Anti-Oxidant Assay

Extracts		Time in minutes						
		0	5	10	15	20	25	30
Aqueous	OD	0.50	0.42	0.39	0.38	0.36	0.35	0.34
	%	55.35	62.50	65.17	66.07	67.85	68.75	69.64
Ethanol	OD	0.61	0.50	0.48	0.46	0.44	0.43	0.41
	%	45.53	55.35	57.14	58.92	60.71	61.60	63.39
Acetone	OD	0.52	0.32	0.28	0.25	0.23	0.23	0.21
	%	53.57	71.42	75.00	77.67	79.46	79.46	81.25
Chloro- form	OD	0.59	0.51	0.49	0.46	0.45	0.44	0.42
	%	47.23	54.46	56.25	58.92	59.82	60.71	62.50
Pet. Ether	OD	0.56	0.51	0.51	0.50	0.50	0.49	0.49
	%	50.00	54.46	54.46	55.35	55.35	56.25	56.25
Control	OD	0.83	0.33	0.24	0.21	0.19	0.17	0.17
	%	25.89	70.53	78.57	81.25	83.03	84.82	84.82

Table 3: OD values of the fractions obtained

Fraction		Time in minutes						
		0	5	10	15	20	25	30
F1	OD	0.49	0.40	0.39	0.39	0.39	0.39	0.39
	%	56.25	64.28	65.17	65.17	65.17	65.17	65.17
F2	OD	0.52	0.51	0.49	0.49	0.49	0.49	0.49
	%	53.57	54.46	56.25	56.25	56.25	56.25	56.25
F3	OD	0.41	0.41	0.39	0.39	0.39	0.39	0.39
	%	63.39	63.39	65.17	65.17	65.17	65.17	65.17
F4	OD	0.45	0.44	0.43	0.42	0.42	0.41	0.41
	%	59.82	60.17	61.60	62.50	62.50	63.39	63.39
F5	OD	0.39	0.38	0.38	0.38	0.38	0.38	0.38
	%	65.17	66.07	66.07	66.07	66.07	66.07	66.07
F6	od	0.47	0.47	0.46	0.46	0.46	0.46	0.46
	%	58.03	58.92	58.92	58.92	58.92	58.92	58.92
F7	OD	0.41	0.41	0.40	0.40	0.40	0.39	0.39
	%	63.39	63.39	64.28	64.28	64.28	65.17	65.17
F8	OD	0.57	0.56	0.56	0.56	0.54	0.54	0.52
	%	63.39	63.39	64.28	64.28	64.28	65.17	65.17
F9	OD	0.39	0.39	0.39	0.39	0.39	0.39	0.39
	%	65.17	65.17	65.17	65.17	65.17	65.17	65.17
Control	OD	0.83	0.33	0.24	0.21	0.19	0.17	0.17
	%	25.89	70.53	78.57	81.25	83.03	84.82	84.82

The quantitative anti-oxidant assay was done and the activity was evaluated for five different extracts. Acetone extract showed maximum anti-oxidant activity of about 81.25% followed by the aqueous extract with 69.64% at 30th minute. The petroleum ether extract showed minimum activity of 56.25%. The acetone extract with maximum antioxidant activity was further analysed using column chromatographic separation. The column chromatography eluted 9 different fractions and the F5 fraction showed higher anti-oxidant activity of 66.07% when compared to other fractions. The minimum antioxidant activity was found to be 56.25% for F2 fraction. Thus the F5 fraction was preferred for antimicrobial activity. The antibacterial activity of *Jasminum azoricum* leaf extract was tested for five different micro-organisms and the zone of inhibition were obtained at different sample concentration. The zone of inhibition analysis at 30 mg/ml showed moderate anti-microbial activity with 20 ± 4 mm for *B.cereus*, *S.aureus* and *Pseudomonas sp.* and for *B.subtilis* the activity was found to be minimum. The antimicrobial activity of similar species belonging to *Oleaceae* family also showed effective results against *Pseudomonas sp.*, *B.cereus* and *B.subtilis*²⁰.

Table 4: Zone of inhibition in diameter

Microorganism	10mg/ml	20mg/ml	30mg/ml	Control (Distilled water)
<i>E.coli</i>	-	12mm	14mm	-
<i>B.subtilis</i>	-	9mm	9mm	-
<i>B.cereus</i>	-	9mm	24mm	-
<i>S.aureus</i>	-	-	20mm	-
<i>Pseudomonas sp.</i>	-	-	17mm	-

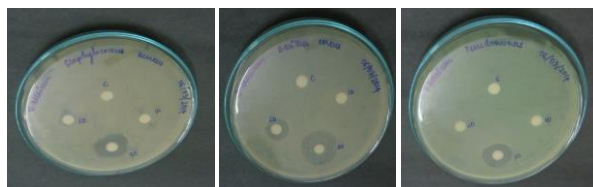


Fig.3: Zone of inhibition obtained for different bacteria (*S.aureus*, *B.cereus*, *Pseudomonas sp.*) at different sample concentrations

CONCLUSION:

The phytochemical analysis revealed the anti-oxidant and anti-microbial activity of *Jasminum azoricum*. The leaf extract of this plant can be used as a potent therapeutically active compound for treating infectious diseases after pharmacological analysis. Thus paves way to development of different modified herbal products for human use.

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