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Studies on phytochemicals and antibacterial potential of *Apluda mutica* L. : An underutilized ethnomedicinal grass

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Abstract

Apluda mutica L. is an underutilized ethnomedicinal grass species that belongs to the Poaceae family. Ethnomedicinally it has been used to treat mouth sores in cattle and dysentery. The main objective of the current research work is to identify the bioactive constituents and *in vitro* antibacterial potential. The qualitative biochemical profiling revealed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins, phenols, glycosides, proteins, and carbohydrates. The LCMS profiling of whole plant methanolic extract showed the existence of 10 bioactive compounds. 2-O-rhamnosyl-swertisin (45.00 %); Sennoside C (27.32 %); Renchangianin B (7.13 %) and Quercetin 3,7-dimethyl ether (6.16 %) were the major identified compounds. the antibacterial activity was evaluated by using an agar well diffusion assay and the results revealed that the methanolic whole plant extract showed moderate antibacterial activity and exhibited the highest inhibition activity against *Enterococcus faecalis* (12.16±0.44 mm), followed by *Staphylococcus aureus* (10.66±0.66 mm) and *Escherichia coli* (07.16±0.44 mm) and the minimum zone of inhibition was showed against *Pseudomonas aeruginosa* (04.66±0.66 mm). Further *in vivo* confirmations are needed to prove the real antibacterial and anticancer efficacy of the test plant *Apluda mutica*.

Key words : *Apluda mutica*, ethnomedicine, phytochemicals, LCMS, antibacterial activity.

Worldwide, infectious diseases caused by bacteria and fungi impact millions of people. Infectious diseases have been a major cause of death and disability throughout human history[10]. Infectious diseases are one of the leading causes of death worldwide and their prevalence is continuously rising. The epidemic of side effects, the difficulty in

creating new agents, the overuse of antibiotics, and, most importantly, the growing problem of resistance all make antimicrobial medication treatments more difficult[12].

Antimicrobial resistance (AMR) is primarily caused by the overuse of antibiotics in both human and veterinary medicine. Antimicrobial resistance (AMR) is the result of prolonged and/or inappropriate exposure to antimicrobial medications, which causes genetic changes in microorganisms (e.g., viruses, bacteria, fungi, and parasites). Poor antibiotic prescribing practices, the use of antibiotics as growth promoters, and the scarcity of substitute antibiotics are the main causes of antimicrobial resistance (AMR). Some major diseases, like gonorrhoea, malaria, and tuberculosis, are becoming futile to treat with the antibiotics that are currently on the market because they are so difficult to treat[20]. Phytomedicines originating from plants have demonstrated significant potential in treating infectious diseases, including opportunistic AIDS infections. Anti-infective drugs have historically been derived from plants; emetine, quinine, and berberine are still very potent antimicrobial substances[7].

In general, more people across the globe are turning to herbal remedies for microbial infections.[5] Research on the antimicrobial qualities of plant extracts and products suggests that plants could be a source of new anti-infective drugs[3,9].

Apluda mutica L. is an underutilized ethnomedicinal plant that belongs to the Poaceae family. Ethnomedicinally it has been previously reported that the whole plant paste was used to treat mouth sores of cattle and also the plant decoction was used to cure dysentery[1,25]. Earlier studies on *Apluda mutica*, concentrated on Anti-diabetic, anti-inflammatory, and anti-oxidant properties[11]. However, the antibacterial properties of *Apluda mutica*, have not been scientifically investigated. The objective of this study is to investigate the phytochemical analysis and antibacterial potential of the methanolic leaf extract of *A. mutica*.

Collection and taxonomical identification of plant materials :

The whole plant samples of *Apluda mutica* were collected near Chikkamagaluru region, Karnataka, India. The collected plant samples were taxonomically identified and voucher specimens were deposited in the herbarium, CARI Bangalore with specimen number (RRCBI-mus 432).

Plant extracts preparations :

The retrieved whole plant materials were washed with tap water, chopped, dried, and pulverized into a coarse powder using a mechanical grinder, and then, Soxhlet extraction was performed with petroleum ether, methanol, and distilled water. The extracted materials were air-dried and stored in airtight containers at 4^{o}C before being used as a test sample for future studies.

Qualitative biochemical profiling :

By using standard protocols [14,15] the collected crude extracts of *Apluda mutica* underwent qualitative phytochemical screening to identify various classes of active phytocons-

tituents including phenols, flavonoids, alkaloids, glycosides, tannins, terpenoids, saponins, steroids, lignins, and carbohydrates.

Quantitative LCMS profiling :

The standard LC-MS model was used to analyze *A. mutica* whole plant methanolic extract. The protocol used was Vijayalakshmi *et al.,*[31].

Instrumentation :

The Acquity H-class UPLC (Waters Corporation, Milford, MA, USA) was employed, which had an integrated vacuum degasser, automatic sample manager (Serial # C10UPA554M, Waters Corporation, Singapore), ultra-performance binary solvent manager (Serial # C10UPB081A, Waters Corporation, Singapore), and injection volume range of up to 100 µL with an optional extension loop. A C18 stationary phase (Accucore C18, 50 x 4.6mm, 2.6µ) was used for chromatographic separation. A Xevo G2-XS QToF (Serial # YFA1548, Waters Corporation, Wilmslow, UK) was employed for mass spectrometric (MS) detection.

LCMS operating details :

The Mobile Phase is made up of 0.1% Formic acid in Water as an aqueous phase (A) and acetonitrile as an Organic modifier (B), and it is delivered at a flow rate of 0.4 mL/min in the following gradient: initially % B is kept at 5% for 1 minute; 1 to 6 minutes (5 to 50% B), 6 to 12 minutes (50 to 95% B), held for 4 minutes, 16 to 17 minutes (95 to 5% B) and held at5% for 3 minute. The sample was injected in a volume of 5 µL. The column oven temperature was kept at an optimal level throughout the chromatographic run (22^{o}C). For MS detection, a positive polarity electrospray ionization (ESI) source was used. The optimal instrument and acquisition parameters were as follows: 50 L/hr. cone gas (nitrogen) flow; 750 L/hr. desolvation gas (nitrogen) flow; 450^{o}C probe temperature; 30 V sampling cone voltage; 150^{o}C source temperature; 80 V source offset voltage; the collision energy ramp varies from 6 eV-50eV (Argon, collision gas), and a mass range of 50 to 2000 m/z. To acquire and process data, Waters Corporation's Mass Lynx software (V4.1, Milford, MA, USA) was used.

In vitro antibacterial activity :

Antibacterial assay was determined using the standard agar well diffusion approach according to Balouiri *et al.,* (2016)[5]. In this method, 24 hours of old cultured bacterial strains on a nutrient broth were swabbed uniformly on solidified nutrient agar (media) plates using sterile cotton swabs. The wells were then punched with a sterilized cork borer to a diameter of 5 mm. Each well of the plate was loaded with 20 µl of methanolic extracts of *A. mutica* at different concentrations. 10 mg of each crude extract were dissolved in 1000 µl of dimethyl sulfoxide (DMSO) and diluted to 100%, 50%, and 25% concentrations, respectively. The standard drug Ciprofloxacin, 1mg/ml, was used as a positive control, and Dimethyl Sulfoxide was used as a negative control. They were added separately into the labeled wells of the plates. The plates were inoculated and incubated at 35-37 degrees Celsius overnight to determine the zone of inhibition. The experiments were replicated three times to obtain average results.

Qualitative biochemical profiling :

The results of qualitative biochemical profiling revealed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins, phenols, glycosides, proteins, and carbohydrates (Table-1). Among the three different solvent extracts methanolic extracts showed the occurrence of the maximum number of phytochemicals followed by aqueous extract and minimum number of phytochemicals.

Qualitative LCMS analysis :

The LCMS chromatogram of methanolic whole plant extract of *Apluda mutica* revealed the existence of 10 bioactive constituents (Figure 1). The identified compounds along with their retention time, peak area percentage, molecular formula, and molecular weights are listed in Table 1.

The results revealed that among the 10 identified bioactive compounds 2-O-rhamnosyl-swertisin (45.00 %) is the major identified constituent followed by Sennoside C (27.32 %), Renchangianin B (7.13 %), Quercetin 3,7-dimethyl ether (6.16 %), Phellopterin (3.50%), Agroastragaloside I (3.14 %), Diosmin (2.83 %), Bryonioside A (1.98 %), and Volkensiflavone (0.74 %).

Table-1. Qualitative analysis of different solvent extracts of *Apluda mutica*

Secondary metabolites	Petroleum ether	Methanol	Aqueous
Alkaloids	+	+	-
Tannins	+	+	+
Glycosides	+	-	-
Phenols	-	+	+
Flavonoids	+	+	+
Sterols	-	+	-
Saponins	-	+	+
Terpenoids	-	+	-
Carbohy-drates	+	+	+
Proteins	-	+	+
-: Negative result; +: Positive results			

Table-2. List of identified bioactive compounds from LCMS analysis of methanolic whole plant extract of *Apluda mutica*

Sl. No	Compound name	Retention time	Peak area %	Molecular formula	Molecular Weight
1.	Myosmine	0.44	2.20	$C_9H_{10}N_2$	146.19
2.	Phellopterin	3.14	3.50	$C_{17}H_{16}O_5$	300.30
3.	Quercetin 3,7-dimethyl ether	5.27	6.16	$C_{17}H_{14}O_7$	330.29
4.	Volkensiflavone	5.93	0.74	$C_{30}H_{20}O_{10}$	540.5
5.	Bryonioside A	9.66	1.98	$C_{36}H_{60}O_9$	636.9
6.	Diosmin	10.22	2.83	$C_{28}H_{32}O_{15}$	608.5
7.	2-O-rhamnosyl-swertisin	10.57	45.00	$C_{28}H_{32}O_{14}$	592.5
8.	Renchangianin B	11.38	7.13	$C_{34}H_{38}O_{11}$	622.7
9.	Sennoside C	12.41	27.32	$C_{42}H_{40}O_{19}$	862.7
10.	Agroastragaloside I	17.09	3.14	$C_{45}H_{74}O_{16}$	871.1

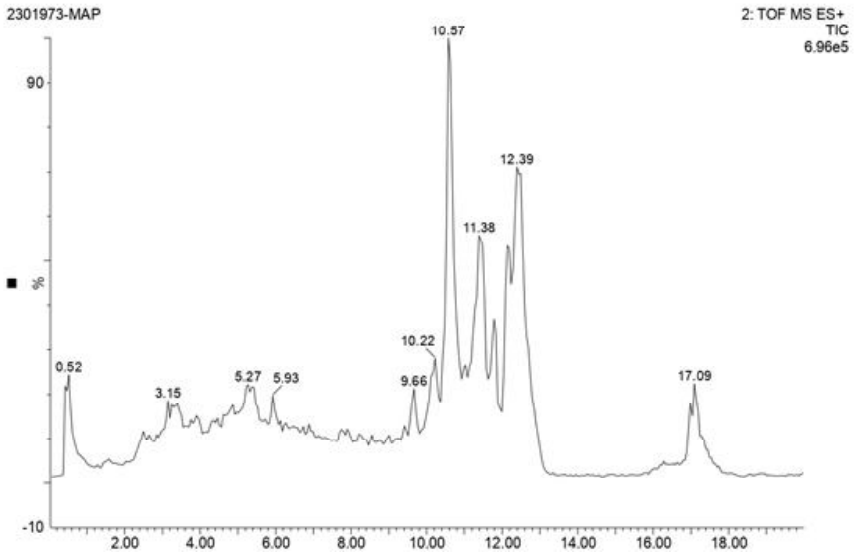

Figure 1: LCMS chromatogram of methanolic whole plant extract of *Apluda mutica* L.

Antibacterial assay :

The antibacterial potential methanolic whole plant extract of *Apluda mutica* L. against four selected pathogenic bacterial strains exhibited an inhibition zone in a dose-dependent manner. The methanolic whole plant extract showed moderate antibacterial activity and exhibited highest inhibition activity against *Enterococcus faecalis* (12.16±0.44 mm), followed by *Staphylococcus aureus* (10.66±0.66 mm) and *Escherichia coli* (07.16±0.44 mm) and the minimum zone of inhibition was showed against *Pseudomonas aeruginosa* (04.66±0.66 mm). The experimental results were triple-checked and results were expressed in M±SEM, (Mean and Standard error of the mean). The zone of inhibition was measured in millimeters (Table-3 and Figure 2).

Table-3. Antibacterial potential of the whole plant extract of *Apluda mutica* L. against pathogenic bacterial strains

Sl No	Name of the bacterial strains	Inhibition zone in mm				
		Concentration in percentage			Standard (Cipro-floxacin)	Control (DMSO)
		100%	50%	25%		
1	*Staphylococcus aureus*	10.66±0.66	07.83±0.60	06.16±0.60	27.33±0.33	00
2	*Enterococcus faecalis*	12.16±0.44	11.83±0.44	09.00±0.57	28.66±0.66	00
3	*Escherichia coli*	07.16±0.44	07.00±0.57	05.83±0.44	27.66±0.33	00
4	*Pseudomonas aeruginosa*	04.66±0.66	03.33±0.60	02.16±0.60	27.00±0.57	00
Note: Values are expressed in Mean ± Standard Error of Mean The experiments were triplicated (n=3)						

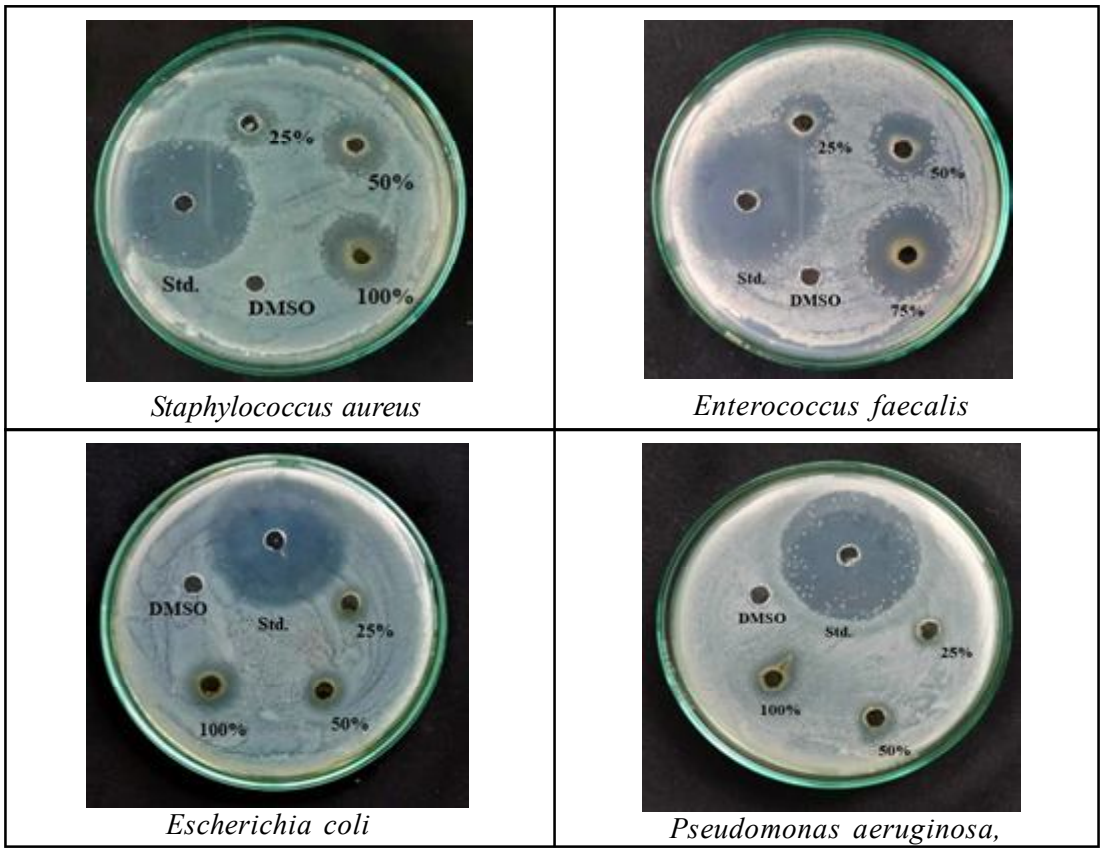

Fig. 2. Antibacterial potential of the whole plant extract of *Apluda mutica* L. against pathogenic bacterial strains.

The current studies on the phytoconstituents of *Apluda mutica* through qualitative analysis have shown the presence of a variety of secondary metabolites in distinct solvent extracts (Table-1). These secondary metabolites have a significant impact on the biological effects of medicinal plants, including antimicrobial, hypoglycemic, antioxidant, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, and anti-leprosy properties[36]. The primary bioactive components are phenolic compounds, which have antimicrobial properties, strengthen the immune system, eliminate free radicals, and regulate gene expression to produce antioxidant activity[23]. Alkaloids are a class of naturally occurring chemicals with pharmacological activity that are crucial to the drug-discovery process. Their pharmacological properties included anti-oxidant, antihyperlipidemic, and anti-obesity properties[28]. Because of their ability to reduce inflammation, protect the heart, and inhibit bacteria, tannins are vital for a variety of biological processes[21]. According to Wang *et al.,* flavonoids are used in diabetes, antimicrobial properties,

anti-inflammatory, and anti-aging drugs[32]. Moreover, saponins have anti-inflammatory, antibacterial, antidote, antifungal, anti-yeast, and antifeedant properties in addition to their hemolytic, cardiotonic, hypotensive, and cardiac depressive actions[27]. In contrast, congestive heart failure and cardiac arrhythmias are treated with glycosides, which are inherently cardioactive medications[22].

The LCMS analysis of methanolic whole plant extract of *Apluda mutica* revealed the existence of 10 bioactive constituents (Figure 1 and Table-2). The identified compound 2-O-rhamnosyl-swertisin was previously isolated from *Echinodorus macrophyllus* was efficient in reducing the hypernociceptive reaction brought on by carrageenan[13] and was also reported to have anti-diabetic properties[33]. The compound Volkensiflavone shows significant antioxidant and antibacterial activities[19]. Another identified bioactive compound Sennoside C recognized to possess a variety of biological functions, such as antioxidant, anti-microbial, and antimutagenic properties[2,24].

The remaining bioactive constituents identified were as follows: Quercetin 3,7-dimethyl ether possesses anti-inflammatory and antibacterial activities[17]. Renchangianin B was reported to possess anti-inflammatory, anticancer, antioxidant, and antibacterial activities[34]. Phellopterin was recognized to possess anticancer antidiabetic properties[6,35]. Further, the compound Myosmine has antioxidant properties[26].

The compound Bryonioside A is cucurbitane glycoside that was previously isolated from the roots of *Bryonia dioica* and reported to have anti-inflammatory and anti-tumor-promoting effects[29]. The compound Agroastragaloside I is unknown for its biological properties.

The antibacterial potential of *Apluda mutica* whole plant methanolic extracts was tested against some selected pathogenic bacterial strains by agar well diffusion assay. The results revealed, that a whole-plant methanolic extract of *Apluda mutica* showed moderate activity and the zone of inhibition is concentration-dependent (Table 3 and Figure 2). The major identified compounds in the LCMS analysis such as Volkensiflavone; Sennoside C; Quercetin 3, 7-dimethyl ether and Renchangianin B have been previously reported to have anti-bacterial properties and also several members of the Poaceae family reported having antibacterial properties[4,8,16,30]. These results show that the methanolic stem extract *Apluda mutica* considerably suppresses the tested bacterial strains.

It is concluded that, *Apluda mutica* has a wide variety of phytochemical constituents. The LCMS screening of whole plant methanolic extract showed the existence of 10 bioactive compounds based on peak area, molecular weight, retention time, and mass spectrum. 2-O-rhamnosyl-swertisin (45.00 %); Sennoside C (27.32 %); Renchangianin B (7.13 %) and Quercetin 3,7-dimethyl ether (6.16 %) were the major identified compounds. manner. The methanolic whole plant extract of *Apluda mutica* showed moderate antibacterial activity. However, additional separation of distinct bioactive phytoconstituents and examination of their antimicrobial properties are necessary to validate the test plant's true medicinal potential.

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