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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL STUDIES ON *CELTIS TIMORENSIS* STEM EXTRACT

Botany

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ABSTRACT

The present paper deals with the phytochemical composition, antioxidant and antibacterial activities of petroleum ether, ethanol, methanol and water extracts of Celtis timorensis stem part. Preliminary phytochemical analysis results revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, and glycosides. Quantitative phytochemical study results revealed that methanol extracts extracted more amount of phenolic content (79.82±3.01 mg GAE/g dwt.). Ammonium molybdate-dependant antioxidant capacity results revealed that ethanol extract of stem part demonstrated higher molybdate-dependant antioxidant (517 mg ASE/g dwt.). The highest DPPH-reducing activity was observed by ethanol and methanol extracts exhibiting the lowest IC50 values (50µg/ml). The highest antibacterial activity was exhibited by water extract followed by ethanol, petroleum ether and methanol extract. Among the tested organisms *Bacillus megatherium* was strongly inhibited by water extract (13mm). *Alkaligenes faecalis* exhibited very feeble activity (8 mm). In conclusion, the stem part extracts of *C. timorensis* rich in total phenolic components exhibited good antioxidant activity and antibacterial activity.

KEYWORDS

Celtis timorensis, total phenolic content, DPPH, Antibacterial activity

INTRODUCTION

Free radicals are very short signalling molecules that play dual roles as toxic and beneficial effects. These are of two types i.e. reactive oxygen and nitrogen species. The imbalance of these molecules plays an important role in the progression of inflammatory diseases. Medicinal plants used in traditional medicinal systems are the major source for the treatment of inflammatory diseases. These plants are rich in a wide variety of chemical constituents such as alkaloids, terpenoids, flavonoids, and tannins and essentials have been found as potent antioxidant components (Lee et al., 2017; Zhang et al., 2015).

Celtis timorensis Spr. is a medium-sized tree of flowering plant belonging to the family Ulmaceae, distributed in India, Nepal, Thailand, Vietnam, and Malesia. Different parts i.e. leaf, stem, root, fruit, etc. of this plant has been used by the tribal community of Indian states to cure various human diseases such as dysentery, jaundice, memory enhancement, toothache, urinary tract food infection (Pullaiah, 2006; Singh et al., 2003; Gouda et al., 2017; Ahmed et al., 2016).

The review on phytochemical composition, antibacterial, antioxidant, and pharmacological studies on *Celtis timorensis* indicated that very few and sporadic attempts are noticed on preliminary phytochemical (Prasanth Kumar et al., 2014), DPPH (Rajaneekar et al., 2013a), antibacterial (Isurika et al., 2019), antidepressant (Rajaneekar et al., 2013b), acute and sub-acute toxicity (Prasanth Kumar et al., 2014) and wound healing activity (Prasanth Kumar et al., 2017) of leaf extracts of *Celtis timorensis*. But no previous report was noticed on qualitative and quantitative phytochemical analysis, molybdate dependant antioxidant, DPPH and antibacterial activity of *Celtis timorensis* stem part. Hence, we selected *Celtis timorensis* stem extracts to evaluate the phytochemical analysis antioxidant and antibacterial studies of five extracts.

MATERIALS AND METHODS

Plant material

Celtis timorensis stem part was collected from the Tirumala hills of Andhra Pradesh. The plant specimen was identified (Voucher #1379) by Dr K. Madhava Chetty, Assistant Professor (Rtd.), Department of Botany, S.V. University, Tirupati.

Preparation Of Plant Extracts

The collected plant samples were washed with distilled water and shade dried at room temperature. Fifty grams of pounded stem material was successively extracted with Petroleum ether (PE), chloroform (CE), ethanol (EE) and methanol (ME) using Soxhlet apparatus for 6-8 hours. The extracts were filtered and concentrated under reduced pressure to dryness and the extracts were used for the assays.

Water Extract Preparation:

One gram of plant material after the successive extraction was taken and soaked in 50 ml distilled water for 24 h and filtered. The filtrate was concentrated in the water bath at 40 ^{0}C and subjected to phytochemical, antioxidant and antibacterial studies.

Preliminary Phytochemical Screening

Qualitative phytochemical tests for alkaloids, saponins, phytosterols, (Auwal et al., 2014), starch, glycosides, phenolic components, gums and tannins (Shaikh & Patil, 2020) were determined using standard methods.

Total Phenolic Content of *Celtis timorensis* Stem Extracts

The total phenolic quantity of *Celtis timorensis* stem extracts was estimated using the methods described (Venkata Ratnam et al., 2015).

Total Antioxidant Capacity of *Celtis timorensis* Stem Extracts

Ammonium molybdate dependant antioxidant capacity of *Celtis timorensis* stem extracts was determined using the methods described (Vishnu Mohan Reddy et al., 2019).

DPPH Reducing Activity

DPPH scavenging capacity of *Celtis timorensis* stem extracts was determined using the methods described (Vishnu Mohan Reddy et al., 2019).

Antibacterial Studies

Microorganisms Used

The microorganisms used in the present study are *Micrococcus luteus* (MTCC 9341), *Arthrobacter protopharmiae* (MTCC 688), *Alkaligenes faecalis* (MTCC 10757), *Enterococcus faecalis* (MTCC 439), *Bacillus megatherium* (MTCC 5981), *Lactobacillus acidophilus* (*MTCC 10307), Salmonella enterica (MTCC 3858) and Pseudomonas aeruginosa* (MTCC 1688) to test the extracts. The organisms were purchased from the MTCC centre, IMTECH, Chandigarh, India.

Antimicrobial Activity:

The antibacterial efficacy of *C. timorensis* stem petroleum ether (PE), ethanol (EE) methanol (ME) and water extracts were studied using the disc diffusion method (Cruikshank et al., 1975). Paper discs (4 mm) impregnated with distinct concentrations of plant extracts were placed on the Petri plates, containing 20ml of Mueller Hinton Agar (MHA) media (Peptone 17.5 g, meat extract 2 g, starch 1.5 g and agar-agar 17 g/L) seeded with 0.1ml of overnight grown microbial suspension. The discs saturated with methanol ethyl acetate and petroleum ether served as negative controls. Bacterial-free zones present around the discs were treated as positive results. The zones were measured after 24 hours and tabulated.

RESULTS & DISCUSSION

Qualitative phytochemical analysis of petroleum ether, ethanol,

methanol and water extract *C. timorensis* stem extracts were assessed using standard procedures. The yield of each extract was depicted in (Table 1). Preliminary phytochemical analysis results revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, and glycosides (Table 2). The critical review of the literature indicated that no previous report was noticed on the preliminary phytochemical composition of *C. timorensis* stem extracts.

Table 1: Yield of *Celtis timorensis* stem extracts

S. No.	Extract Type	Yield (%)
Stem Extracts		
1	Petroleum ether	1.74%
2	Chloroform	0.6%
3	Ethanol	2.72%
4	Methanol	2.62%
5	Water	3.0%

Table 2: Preliminary phytochemical analysis of *Celtis timorensis* Stem extracts

Type of component	PE	CE	EE	ME	WE
Alkaloids	NR	NR	NR	+	NR
Saponins	NR	NR	NR	NR	+
Phytosterols	NR	NR	NR	NR	NR
Phenolic components	NR	+	+	+	NR
Tannins	+	+	+	+	+
Flavonoids	+	NR	NR	NR	NR
Terpenoids	NR	+	+	+	+
Glycosides	NR	+	+	+	+
Gums and mucilages	NR	NR	NR	NR	NR

Total phenolic contents of petroleum ether, chloroform, ethanol, methanol and water extracts of *C. timorensis* stem were estimated using the Folin Ciocalteau reagent method. Gallic acid was used as the standard component and the amount of total phenolic components was expressed in milligrams of Gallic acid equivalents per gram dry weight (mg GAE/g dwt.). The results revealed that the total phenolic content of stem part extracts revealed that methanol extract extracted more amount of phenolic content (79.82±3.01 mg GAE/g dwt.), while petroleum ether extract have very less amount (18.99±0.24 mg GAE/g dwt.) (Figure1). This may be due to the solubility of phenolic components in methanol. Higher amounts of the total phenolic content of methanol extract of different medicinal plant parts were reported and stated that methanol solvent is efficient for extraction of a good amount of phenolic components (Boeing et al., 2014; Santas et al., 2008; Dieu-Hien Truong et al., 2019). The review of the literature showed that no previous report was noticed on the total phenolic content of water, petroleum ether, chloroform, ethanol and methanol extracts of the stem part.

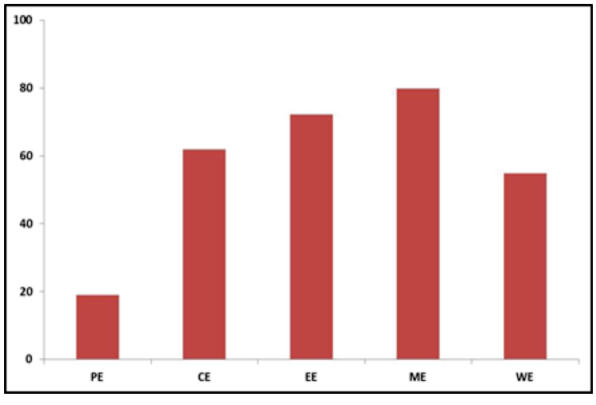

Figure 1. Total Phenolic content of *Celtis timorensis* stem extracts

The stem part extracts exhibited antioxidant capacity between 133 to 517 mg ASE/g dwt. Ethanol extract of the stem part demonstrated higher molybdate-dependant antioxidant (517 mg ASE/g dwt.) and petroleum ether extract showed lower antioxidant capacity (133.0±1.08) (Figure 2). The higher antioxidant capacity of ethanol extract from medicinal plants has been reported (Do et al., 2014; Cho et al., 2010; Ashafa et al., 2010). The higher amount of antioxidant capacity of ethanol extract of the stem part of *C. timorensis* may be due to the high amount of total phenolic components. The results were correlated with the phenolic content of extracts of stem parts. A similar type of correlation of total phenolic content with total antioxidant activity was reported by several researchers (Yu et al., 2021; Reddy & Grace, 2016; Neethu Joy & Mahesh Mohan, 2020). The review of the literature indicated that no previous report was noticed on molybdate dependant antioxidant capacity of petroleum ether, ethanol, chloroform, methanol and water extracts of *C. timorensis* stem parts.

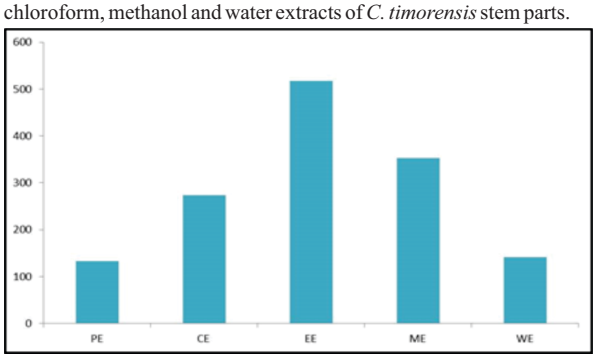

Figure 2 Total Antioxidant Capacity of *Celtis timorensis* stem extracts

DPPH scavenging activity of petroleum ether, chloroform, ethanol, methanol and water extracts of *C. timorensis* stem part showed concentration dependant DPPH scavenging activity. The highest DPPH-reducing activity (>90%) was observed by chloroform, ethanol and water extracts of the stem part. While petroleum ether extract failed to reduce DPPH purple colour. Ethanol and methanol extracts exhibited the lowest IC_{50} values (50µg/ml). The strong DPPH reducing the activity of ethanol and methanol extracts of the stem part may be due to the presence of a higher amount of total phenolic content. Several researchers have reported strong DPPH quenching capacity of methanol or ethanol extracts of medicinal plants (Nandakumar & Indumathi, 2013; Khan et al., 2013; Adebiyi et al., 2017; Ravishankar and Indira, 2012). No previous report was noticed on the DPPH scavenging activity of stem chloroform, ethanol, methanol and water extracts. This is the first report on the DPPH scavenging activity of *C. timorensis* stem extracts.

The antibacterial activity of petroleum ether, chloroform, ethanol, methanol and water extracts of the stem part of *C. timorensis* revealed that all the tested pathogens exhibited concentration dependant antibacterial activity (Tables 3 to 6). The highest antibacterial activity was exhibited by water extract followed by ethanol, petroleum ether and methanol extract. Among the tested organisms gram-positive bacterial species *Bacillus megatherium* was strongly inhibited by water extract (13mm) followed by *M. luteus, A. protopharmiae, E. faecalis, L. acidophilus* and *P. aeruginosa* (12mm each) respectively. *Alkaligenes faecalis* exhibited very feeble activity (8 mm). Reaming tested organisms expressed very feeble antibacterial activity by all the tested extracts. The strong antibacterial activity of water and ethanol extracts of the stem part of *C. timorensis* may be due to the presence of a good amount of phenolic components. The phenolic components exhibit their antibacterial action in many ways. It can cause morphological changes in bacterial cells i.e. shapes, wrinkles on the cell membrane and damage cell membrane in both outer and inner membranes. The mechanism of antibacterial activity of polyphenols is closely related to the chemical nature, and position of hydroxyl and methyl groups (Efenberger-Szmechtyk et al., 2020; Hayriye Cetin-Karaca, 2011; Natalia Vaou et al., 2021).

Table 3. Antibacterial activity of Celtis timorensis Stem Petroleum ether extract

Microorganism	Petroleum Ether extract Concentration (µg/Disc*) Zone of Inhibition (mm)			
	50*	100*	150*	300*
Micrococcus luteus (MTCC 9341),	5.67±0.76	6.50±1.0	7.17±0.57	7.50±0.5
Arthrobacter protopharmiae (MTCC 688),	6.67±0.76	7.17±0.57	7.50±0.5	8.33±0.28
Alkaligenes faecalis (MTCC 10757)	5.33±0.57	7.17±0.57	9.33±0.28	11.05±0.5
Enterococcus faecalis (MTCC 439),	6.50±1.0	6.83±0.57	7.50±0.5	10.67±0.28
Bacillus megatherium (MTCC 5981)	5.67±0.76	8.33±0.28	9.17±0.28	9.33±0.28
Lactobacillus acidophilus (MTCC 10307)	6.67±0.76	6.83±0.57	8.17±0.57	11.33±0.28

Salmonella enterica (MTCC 3858)	5.67±0.76	7.17±0.57	8.33±0.28	9.17±0.28
Pseudomonas aeruginosa (MTCC 1688)	5.50±1.0	6.50±1.0	8.17±0.57	11.33±0.28

Table 4. Antibacterial activity of *Celtis timorensis* Stem Ethanol extract

Microorganism	Ethanol extract Concentration (µg/Disc) Zone of Inhibition (mm)			
	50	100	150	300
Micrococcus luteus (MTCC 9341),	5.67±0.76	7.17±0.57	8.33±0.28	10.67±0.28
Arthrobacter protopharmiae (MTCC 688),	6.50±1.0	8.33±0.28	9.17±0.28	12.33±0.28
Alkaligenes faecalis (MTCC 10757)	7.17±0.57	8.33±0.28	10.67±0.28	11.05±0.5
Enterococcus faecalis (MTCC 439),	5.33±0.57	7.17±0.57	9.33±0.28	12.33±0.28
Bacillus megatherium (MTCC 5981)	7.17±0.57	9.33±0.28	11.05±0.5	11.33±0.28
Lactobacillus acidophilus (MTCC 10307)	7.17±0.57	9.17±0.28	9.33±0.28	10.67±0.28
Salmonella enterica (MTCC 3858)	6.83±0.57	7.50±0.5	8.17±0.57	12.33±0.28
Pseudomonas aeruginosa (MTCC 1688)	7.17±0.57	9.17±0.28	12.33±0.28	12.33±0.28

Table 5. Antibacterial activity of *Celtis timorensis* stem Methanol extract

Microorganism	Methanol extract Concentration (µg/Disc) Zone of Inhibition (mm)			
	50	100	150	300
Micrococcus luteus (MTCC 9341),	5.33±0.57	5.67±0.76	6.50±1.0	7.17±0.57
Arthrobacter protopharmiae (MTCC 688),	5.33±0.57	5.67±0.76	6.83±0.57	7.50±0.5
Alkaligenes faecalis (MTCC 10757)	7.17±0.57	8.33±0.28	9.17±0.28	9.33±0.28
Enterococcus faecalis (MTCC 439),	6.83±0.57	7.50±0.5	8.17±0.57	9.33±0.28
Bacillus megatherium (MTCC 5981)	5.67±0.76	6.50±1.0	9.17±0.28	11.05±0.5
Lactobacillus acidophilus (MTCC 10307)	6.50±1.0	7.17±0.57	8.17±0.57	9.33±0.28
Salmonella enterica (MTCC 3858)	5.33±0.57	5.67±0.76	6.83±0.57	7.50±0.5
Pseudomonas aeruginosa (MTCC 1688)	6.83±0.57	7.50±0.5	8.33±0.28	10.67±0.28

Table 6. Antibacterial activity of *Celtis timorensis* stem water extract

Microorganism	Water extract Concentration (µg/Disc) Zone of Inhibition (mm)			
	50	100	150	300
Micrococcus luteus (MTCC 9341),	7.17±0.57	8.17±0.57	9.33±0.28	12.33±0.28
Arthrobacter protopharmiae (MTCC 688),	6.50±1.0	7.17±0.57	10.67±0.28	12.33±0.28
Alkaligenes faecalis (MTCC 10757)	6.50±1.0	6.83±0.57	7.50±0.5	8.17±0.57
Enterococcus faecalis (MTCC 439),	6.83±0.57	7.50±0.5	9.33±0.28	12.33±0.28
Bacillus megatherium (MTCC 5981)	8.33±0.28	10.67±0.28	11.05±0.5	13.17±0.28
Lactobacillus acidophilus (MTCC 10307)	7.50±0.5	9.33±0.28	10.67±0.28	12.33±0.28
Salmonella enterica (MTCC 3858)	8.17±0.57	9.33±0.28	10.67±0.28	12.33±0.28
Pseudomonas aeruginosa (MTCC 1688)	8.17±0.57	9.33±0.28	10.67±0.28	12.33±0.28

CONCLUSION

In the present investigation, *Celtis timorensis* stem part extracts demonstrated a good amount of phytochemical composition. The extracts exhibited strong antioxidant capacity against DPPH scavenging and a good amount of phosphomolybdenum-dependent antioxidant capacity. The extracts strongly inhibited human pathogens in vitro conditions. The research findings of the present study suggest that the extracts of *C. timorensis* could be used as a potential source of natural antioxidant/ antibacterial components. It is further required to isolate the active principle and validate its pharmacological properties *in vivo* models.

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