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A preliminary phytochemical and antimicrobial analysis on *senna alata* (L.) Roxb. (Leguminosae)

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Abstract
The current investigation was carried out to establish the phytochemical and antimicrobial profile of the leaves, flowers, and fruits of *Senna alata* (L.) Roxb. (Leguminosae), aiming to determine its characteristics for identification and prevent taxonomic confusion with other species within the same genus. The study encompassed preliminary organoleptic, phytochemical, and antimicrobial analyses. The physical attributes of various solvent extracts revealed the color, scent, and texture of the powdered leaves, flowers, and fruits. The initial phytochemical analysis confirmed the presence or absence of alkaloids, saponins, tannins, carbohydrates, flavonoids, phytosterol, and phenolic acid in the leaves, flowers, and fruits. Ultimately, the antimicrobial activity of the acetone extracts was found to be lower compared to the ethanol extracts. The ethanol extract of *Senna alata* leaves exhibited the highest activity against *Bacillus* sp. These findings can be utilized to verify the authenticity of *Senna alata* leaves, flowers, and fruits, ensuring their proper identification and standardization for the collection of raw plants used in herbal drug preparation. The use of leaf extracts with recognized antimicrobial properties holds significant potential for therapeutic treatments.

Keywords: *Senna alata*, leaves, flowers and fruits, phytochemical, antimicrobial activity

Introduction
Senna alata, commonly known as the candle bush, emperor's candlesticks or ringworm shrub, is a flowering plant native to tropical regions of Central and South America, as well as certain parts of Africa and Asia. It belongs to the Fabaceae family and is closely related to other species of Senna.
Senna alata is a perennial shrub that can grow up to 3 meters (10 feet) in height. It has a woody stem with multiple branches, and its leaves are compound, large, and arranged alternately along the stems. Each leaf consists of several pairs of elliptical leaflets with a prominent central vein and a smooth or slightly serrated margin. The flowers are bright yellow and borne in large clusters at the ends of the branches. Each individual flower has five petals, with the uppermost one forming a distinctive" candlestick" shape, giving the plant its common name. It is produces elongated seed pods that contain numerous small, flat seeds. The pods turn brown as they mature and eventually split open, releasing the seeds. These seeds have a hard coating that allows them to survive harsh conditions and remain viable for a long time (Nadkami and Nadkami, 1982) [1]. Aside from its ornamental value, *Senna alata* has been traditionally used in various cultures for its medicinal properties. It's important to note that while *Senna alata* has medicinal potential. The leaves have been reported to be diuretic and purgative (Chopra *et al* 1986) [2].
The leaves have been utilized for the management of bronchitis and asthma, constipation, ringworm skin ailments, anti-venom properties, and as an abortifacient, exhibiting acknowledged antimicrobial activity (Bhat *et al*., 1990) [3]. The plant has been reported to possess therapeutic properties for the treatment of dysentery, scabies, ulcers, helminthic infection, and stomach disorders (Abubacker and Kumar, 2007; Doughari and Okafor, 2007) [4, 5]. *Senna alata* leaf extract has been documented to exhibit various pharmacological activities, including: Antibacterial effects (Muthuselvam *et al*, 2016; Tatsimo *et al*, 2017) [6, 7]. Cytotoxicity (Olarte *et al*, 2013; Raji *et al*, 2015) [8, 9]. Anti-inflammatory properties (Lewis *et al*, 2011) [10]. Antidiabetic effects (Laishram *et al*, 2016) [11]. Antihepatotoxic and hepatoprotective effects (Neharkar and Gaikwad, 2011) [12]. Antiviral properties (Shaheen *et al*, 2015) [14]. Strong DPPH radical scavenging activities (Chatterjee *et al*, 2013) [13]. Natural plant compounds exhibit significant structural diversity. Nonetheless, there has been a growing focus on extracts and biologically active compounds derived from plant species used in herbal medicine due to their fewer side effects. Medicinal plants play a crucial role as anticancer agents, and it is noteworthy that many currently used anticancer agents are sourced from plants (Kumar *et al*, 2011) [15].
The current research aims to investigate the phytochemical characterization and the antibacterial properties of various solvent fractions for the quality control of the herbal drug.

Material methods
Plant sample collection
The robust plants of *Senna alata* were gathered from the Kathakkinaru village in Madurai district (Latitude 100 05' N and Longitude 780 16' E). The specimens were recognized utilizing" The Flora of the Tamilnadu Carnatic"- Part-1 (Mathew, 1983) [16]. Vigorous leaves, blossoms, and fruits of the plants were chosen, detached, and the

components of the plants were rinsed individually with tap water and air-dried indoors at ambient temperature until a constant weight was achieved. The air-dried samples were pulverized in an electric blender and stored in plastic bags for future utilization.

Phytochemical analysis

The ground foliage, blossoms, and fruits of S. alata were examined to assess the physical attributes, including hue, fragrance, and texture of diverse soluble extracts. The mature and disease-free leaves and flowers were air-dried to maintain the integrity of their chemical constituents. Subsequently, the specimen was pulverized using an electronic blender, and phytochemical research was conducted as per the methodology proposed by Kokate (2000) [17].

Preparation of plant extract

Measured 10 grams of plant material and Perform the extraction on the plant material using organic solvents such as ethanol, petroleum ether, and acetone (100 mL) in a mechanical shaker equipped with temperature control (maintained at room temperature) and set at a constant stirring speed of 200 revolutions per minute (rpm). Allow the sample to sit for 24 hours, then filter it using Whatman No. 1 filter paper. Repeat the extraction process three times, followed by concentration under vacuum at 40°C utilizing a rotary evaporator. Finally, store the concentrated material at 4°C for future use. (Chandran *et al*., 2012) [18]

Test organisms

The extracts were evaluated on the subsequent two gram-positive bacteria: *Bacillus subtilis*, and *Pseudomonas*, as well as gram-negative bacteria such as *Escherichia coli*. All the strains were acquired from the Department of Microbiology, Thiagarajar College, Madurai, Tamil Nadu.

Anti bacterial assay

The plant extracts that were chosen underwent testing to determine their antibacterial activity using the agar disc diffusion assay, following the methodology established by Bauer *et al*. (1966) [19]. Nutrient agar was used to test for antibacterial activity, while Potato Dextrose Agar medium was employed to assess antifungal activity. Initially, 5 mL of overnight inoculum was prepared using nutrient broth and then treated separately with various concentrations of the plant sample. Positive and negative controls were maintained separately. After the treatment, bacterial cells were harvested by centrifugation at 5000 × g for 10 minutes. The bacterial cells were swabbed onto autoclaved glass slides and fixed with 2.5% glutaraldehyde for 2 minutes. Subsequently, the slides were gradually washed with 70-100% ethanol and left to air-dry. Finally, the slides were sputter-coated with gold palladium under vacuum and examined using scanning electron microscopy (SEM) according to Shi *et al*. (1996) [20].

Results

Macroscopical and organoleptical characters

In the present investigation, the leaves of S. alata were observed to have the following characteristics: smooth surface, oblong-elliptic shape, entire margin, obtuse apex, asymmetrical base, simple venation, and orange flowers with a glabrous appearance.

The powdered leaf exhibited the following organoleptic properties like dark green color, odor, slightly bitter taste, gritty texture, and even coarse particles when dissolved in ethanol and petroleum ether. However, when the leaves were extracted with acetone, they displayed a yellowish-green color, characteristic odor, and even coarse particles. The ethanol-extracted flower appeared dark orange-brown and had a characteristic odor, bland taste, gritty texture, and was not sticky. On the other hand, the flower extracts obtained using acetone and petroleum ether showed a yellowish-orange color. Regarding the fruit, it was predicted to be dark brown and brown in color, with a dirty odor and uneven coarse particles when subjected to ethanol, acetone, and petroleum ether extraction.

Fig 1

The extract's phytochemical profile is displayed in tables 1 and 2. In the case of *Senna alata* flower, the following compounds were extracted using ethanol compared to the other two solvents: alkaloids, saponins, carbohydrates, phenols, tannins, and flavonoids. However, alkaloids were present in all the extracts, including ethanol, acetone, and petroleum ether. The ethanolic extracts contained alkaloids, saponins, carbohydrates, phytosterols, phenols, tannins, and flavonoids. Acetone and petroleum ether extracts of *Senna alata* fruit revealed the presence of alkaloids, phytosterols, and flavonoids.

The antimicrobial activity of *Senna alata* leaf and fruit was examined against Gram-negative Escherichia coli, Pseudomonas, and Gram-positive Bacillus at various concentrations (10%, 20%, 30%, and 40% b/v). The antibacterial activity was assessed by measuring the zone of inhibition of bacterial growth. The results of the zone of inhibition against Escherichia coli, Pseudomonas, and Bacillus are shown in tables 3 and 4, as well as in figures 1 and 4. The findings indicated that the fractions with the strongest activity were ethanol, followed by acetone, while petroleum ether exhibited the least activity (Figure 3). The S. alata fruit extract with ethanol demonstrated the highest zone of inhibition, measuring 7.33 ± 2.51 mm against Bacillus. The S. alata flower extract with acetone exhibited a zone of inhibition of 6 ± 2.5 mm against Escherichia coli. The ethanol extract of S. alata flower also displayed significant inhibitory effects against Bacillus, measuring 5.33 ± 0.67 mm.

Table 1: Percentage of dry weight and physical characters of the leaves, flower and fruit of *Senna alata* L.

S.No	Leaves extract	Crude compound weight (g)	Colour	Odour	Consistency
leaves of *Senna alata*					
1	Ethanol	1.5	Dark green	Characteristic	even coarse particles
2	Acetone	4.3	Yellowish green	Characteristic	even coarse particles
3	Pet ether	2.5	Dark green	Characteristic	even coarse particles
flower of *Senna alata*					
1	Ethanol	3.6	Dark orange brown	Aromatic	Not Sticky
2	Acetone	3.2	Yellowish orange	Aromatic	Not Sticky
3	Pet ether	1.3	Yellowish orange	Aromatic	Not Sticky
fruit of *Senna alata*					
1	Ethanol	3.9	Dark brown	Dirty odour	uneven coarse particles
2	Acetone	2.6	Brown	Dirty odour	uneven coarse particles
3	Pet ether	1.9	Brown	Dirty odour	uneven coarse particles

Table 2: Phytochemical screening of *Senna alata* Leaf

S. No	Phytochemicals	Test	Solvents used		
			Ethanol	Acetone	Petroleum ether
1	Alkaloids	Mayer	+	+	+
		Wagner	+	+	+
2	Saponine	Foam	+	+	-
3	Carbohydrate	Molish's test	+	-	-
4	Phytosterol	Salkowski	-	-	-
		Liberman	-	-	-
5	Phenol	Lead acetate test	+	-	-
6	Tannin	Gelatin	+	-	-
7	Flavonoids	Alkaline reagent test	+	+	+

(+)=Present, (-)=absent

Table 3: Phytochemical screening of *Senna alata* flower

S. No	Phytochemicals	Test	Solvents used		
			Ethanol	Acetone	Petroleum ether
1	Alkaloids	Mayer	+	-	+
		Wagner	+	+	+
2	Saponine	Frothing test	-	+	-
3	Carbohydrate	Molish's test	-	-	-
4	Phytosterol	Salkowski	-	-	-
		Liberman	+	+	+
5	Phenol	Lead acetate test	+	-	-
6	Tannin	Gelatin	+	-	-
7	Flavonoids	Alkaline reagent test	+	-	-

(+)=Present, (-)=absent

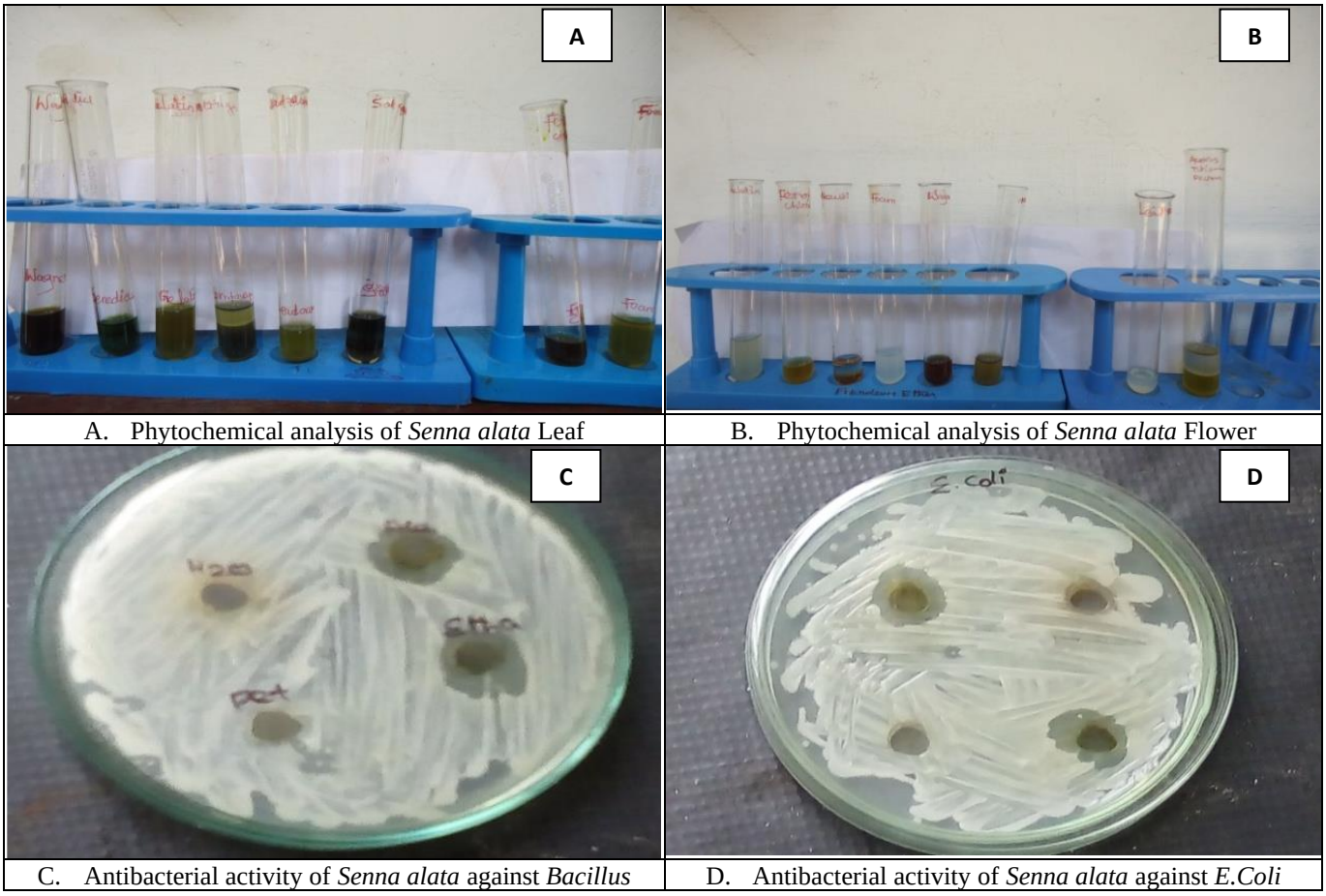
Table 4: Phytochemical screening of *Senna alata* Fruit

S.No	Phytochemicals	Test	Solvents used		
			Ethanol	Acetone	Petroleum ether
1	Alkaloids	Mayer	+	-	+
		Wagner	+	+	+
2	Saponine	Frothing test	-	+	-
3	Carbohydrate	Molish's test	-	+	-
4	Phytosterol	Salkowski	-	-	-
		Liberman	-	-	-
5	Phenol	Lead acetate test	-	-	-
6	Tannin	Gelatin	+	+	+
7	Flavonoids	Alkaline reagent test	-	+	-

(+)=Present, (-)=absent

Table 5: Antibacterial activity of *Senna alata* (Inhibition zone in mm) *C. alata Leaf*

Organism	Solvents used		
Ethanol	Acetone		Petroleum ether
Bacillus	7.33 ± 2.51	4.66 ± 1.1	-
E. coli	NI	5.27 ± 1.8	4 ± 1.1
Pseudomonas	4.66 ± 0.57	3.59 ± 0.9	-
C. alata Flowr			
Bacillus	5.33 ± 0.67	3.21 ± 1.1	-
E. coli	3.11 ± 0.8	6 ± 2.5	-
Pseudomonas	NI	4.34 ± 1.3	-



A. Phytochemical analysis of *Senna alata* Leaf

B. Phytochemical analysis of *Senna alata* Flower

C. Antibacterial activity of *Senna alata* against *Bacillus*

D. Antibacterial activity of *Senna alata* against *E.Coli*

Fig 2

Discussion

In our current investigation findings, the majority of the phytochemicals, including Alkaloids, Saponine, Carbohydrate, Phytosterol, phenols, tannins, and flavonoids, were anticipated in all solvents. A diverse array of phytochemicals were observed to be present in ethanol compared to the other two solvents, both in the flower and fruit of C. alata. Similar outcomes have been reported by several researchers. Sujatha and S. Asokan (2017) [21] deduced that the hexane leaf extract of *Senna alata* exhibited a variety of phytocompounds, such as carbohydrates, tannin, phenol, saponin, flavonoid, steroid, terpenoids, glycosides, and alkaloids. Among them, glycosides, quinines, saponins, phenols, flavonoids, and steroids were identified. In the case of Cassia fistula L. barks, ethanolic extracts were found to contain tannins, flavonoids, polyphenols, saponins, triterpenoids, and anthraquinones (Chaerunisaa *et al*., 2018). The higher solubility of the active components in an organic solvent like ethanol may explain these observations (Lin *et al*., 1999) [23].

Our findings also demonstrated that the flower and fruit extracts of ethanol and acetone acted as effective barriers against all the microorganisms studied. However, the greatest inhibitory effect was observed against Bacillus and E. coli. Aiyelaagbe *et al*. (2007) [24] documented that the root extract of J. curcas, containing certain secondary metabolites, inhibited microorganisms associated with sexually transmitted infections. Additionally, Neumann *et al*. (2004) [25] provided evidence for the antiviral properties of teroids. The stem bark extracts of J. curcas, which contain flavonoids, exhibited a broad spectrum of biological activities, including antimicrobial effects. The antimicrobial activity of the plant extracts, as demonstrated by Escalona-Arranz *et al*. (2010) [26] and Desta (1993) [27], can be characterized by various mechanisms. These studies have indicated that Gram-negative organisms display relatively lower susceptibility to the herbal extracts compared to Gram-positive isolates, suggesting the possible presence of broad spectrum antibiotic compounds. The tannins found in tamarind pulp exert their antimicrobial action by inactivating microbial adhesions, enzymes, and cell envelope proteins. Additionally, they are known to form complexes with microbial polysaccharides (Cowan, 1999) [28].

Amidst the gram-positive and gram-negative bacteria tested, the gram-negative bacteria exhibited greater susceptibility to the extracts. This antibacterial activity can lead to membrane expansion, enhanced membrane fluidity and permeability, disruption of membrane-embedded proteins, inhibition of respiration, and alteration of ion transport processes in both Gram-positive and Gram-negative bacteria, as stated by Carson *et al*. (2002) [29], Brehm-Stecher and Johnson (2003) [30], and Trombetta *et al*. (2005) [31]. Plant alkaloids, such as berberine found in Berberis species and piperine found in Piper species, have the ability to interact with the bacterial cytoplasmic membrane, intercalate with DNA, and inhibit efflux pumps in Staphylococcus aureus (Khan *et al*., 2006) [32]. Phenols and phenolic acids can induce energy production disruption through enzyme inhibition by oxidized products, reactions with sulfhydryl groups, or through more nonspecific interactions with proteins (Mason and Wasserman, 1987) [33].

Phenolic extracts from Origanum vulgare and Vaccinum macrocarpon resulted in urease suppression and disruption

of energy production by inhibiting proline dehydrogenase at the plasma membrane of the Gram-negative human gastric pathogen Helicobacter pylori (Lin *et al*, 2005) [23]. This flavonoid was also observed to enhance permeability of the inner bacterial membrane and dissipate the membrane potential (Mirzoeva *et al* 1997) [34]. Glycoside saponins may induce pore-like structures that modify membrane permeability, leading to changes in the ionic balance between intracellular and extracellular compartments (Melzig *et al* 2001) [35].

Cellular deterioration, disruption of membrane structure, release of intracellular contents, cytoplasmic coagulation, and depletion of proton motive force can lead to cell death (Burt, 2004; Tiwari *et al*., 2009) [36, 37]. The efficacy of antimicrobial compounds depends on the food's pH, the type and quantity of contaminating microorganisms, and the type and concentration of the antimicrobial agent. The effectiveness of antimicrobials may also be influenced by storage temperature, as the diffusibility of compounds is temperature-dependent (Friedman *et al*., 2004) [38]. Phenolic compounds likely exert their toxic effects at the membrane level, as a strong correlation between the toxicity and hydrophobicity of various phenolic compounds has been observed (Sierra-Alvarez and Lettinga, 1991) [39]. Phenol alters membrane function and affects the protein-to-lipid ratios in the membrane (Keweloh *et al*., 1990) [40], leading to the efflux of potassium ions (Heipieper *et al*., 1991) [41]. Catechins have been shown to disrupt membrane integrity by causing leakage from liposomes (Ikigai *et al*., 1993) [42].

Conclusion

Evidently, despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug-resistant microorganisms pose an enormous threat to public health. The utilization of medicinal plants with antimicrobial properties demands greater attention to address the unhygienic situation. The demonstration of the antimicrobial activity of *Senna alata* in this research provides scientific knowledge for its application as a health remedy in rural communities. Further research is necessary to investigate the activity of the extracts against a broader range of bacteria and fungi, as well as to study the toxicology and refine the extraction process to isolate the pure active constituents. We anticipate that the findings of this study will serve for future investigations that may lead to the incorporation of the active components of *Senna alata* in drug preparation in the near future.

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