Utility assessment of Singapore daisy as a tool for management

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ABSTRACT

Singapore daisy is a perennial invasive weed of Kerala having the potential to adversely affect environment and biodiversity. One of the possible methods for the management of invasive weeds is through their utilisation. The study explored the potential of Singapore daisy for phytoremediation of contaminated soils along with its potential as a fodder crop. The results revealed that the invasive plant has the potential for phytoextraction of cadmium and lead; phytostabilisation of cadmium, arsenic, chromium and lead. Analysis of proximate principles of Singapore daisy revealed that the nutritive value was comparable to that of common fodder crops cultivated in Kerala. However, since the plant has phytoremediation potential it cannot be recommended as a fodder crop unless it is growing in uncontaminated soil.

Keywords: [Singapore daisy, Sphagneticola trilobata, Phytoremediation, Utilisation}

1. INTRODUCTION

Singapore daisy (*Sphagneticola trilobata*), is a perennial weed belonging to Asteraceae family, under the order Asterales, which is the subfamily of Asteroideae and a tribe of Heliantheae (Balekar *et al*., 2014; Prasanna et al., 2019). It is reported as one among the hundred world's worst invasive species by IUCN (2024). Other common names include wedelia, yellow creeping daisy, creeping daisy, trailing daisy and rabbit's paw. Singapore daisy is a native of Central America, Mexico, South America and West Indies (Chi *et al.,* 2021). It is a creeping, perennial mat forming herb having the ability to form thick carpet over the soil. It is typically introduced as an ornamental plant in different parts of the world, owing to its lush green leaves and vivid yellow blooms (Azeem *et al*., 2020).
Sphagneticola trilobata has long been used as a traditional herbal medicine in South America, China, Japan, and India to treat a variety of illnesses. The aerial parts of this plant are used in traditional medicine in the Caribbean and Central America to treat bronchitis, colds, abdominal pain, and dysmenorrhea; folk medicine uses it to treat backaches, muscle cramps, rheumatism, stubborn wounds, sores, swellings, and arthritic aching joints (Balekar et al., 2014). Singapore daisy can be utilised as a source of organic nutrition due to its high nitrogen content (Simarmata *et al.,* 2016). The plant has anti-inflammatory, anti-microbial, analgesic, anti-oxidant, hepatoprotective and anti-diabetic properties; several phytoconstituents have been isolated and identified from various plant parts. The stem, leaves, and roots of *Sphagneticola trilobata* has anti-inflammatory, anti-microbial, analgesic and anti-oxidant activities (Prasanna *et al*., 2019). Methanolic extract from *Sphagneticola trilobata* leaves inhibited MCF-7 breast cancer cell lines, owing to its modest antioxidant activity. Furthermore, it inhibits Gram- negative bacteria like *Escherichia coli* and *Salmonella typhi* with comparable efficacy to commercial medications such as tetracycline, clindamycin,

ciprofloxacin, ofloxacin, chloramphenicol, and ampicillin (Mardina *et al*., 2021). It is also found to have anti-diabetic, cytotoxic, antiproliferative, larvicidal, anti-leishmaniasis, anti-oxidant and antipyretic activities (Sethu *et al*., 2023).

Singapore daisy can be used as a phytoremediator to treat waste effluence and contaminated environment with heavy metals (Sandoval and Rodriguez, 2013). The flexibility in using the weed biomass opens up a variety of options for managing them sustainably (Sharma and Pant, 2019). Rachmadiarti *et al*. (2019) revealed that *Sphagneticola trilobata* was capable of absorbing lead and another study revealed that the plant can accumulate more than 100 mg/kg of Cadmium and has tolerance mechanism that makes it a viable candidate for phytoremediation of this element (Pernia *et al.,* 2019). Aveiga *et al.* (2023) evaluated the phytoremediation capacity of *Sphagneticola trilobata* for Al, Cd, Ag, Cr, Ga and Sr. The roots accumulated 27 % more Cd than shoots and also had high potential of phytoextraction and bioaccumulation of Cd, Ag, Sr and Ga.Several invasive weeds like *Parthenium hysterophorus*, *Mikania micrantha*, *Chromolaena odorata, Mimosa invisa and Lanatana camara* are utilised as green manure in several parts of the world (Raj and Syriac, 2016).

2. MATERIAL AND METHODS

The study was conducted in Kasaragod district of Kerala (India). Three locations namely *Kalichampothi* (Latitude: 12.313313^{o}, Longitude: 75.133358^{o}), *Nileshwaram* (Latitude: 12.257588^{o}, Longitude: 75.119024^{o}) and *Azhithala* (Latitude: 12.203972^{o}, Longitude: 75.119429^{o}) were chosen for the study. The biomass production of *Sphagneticola trilobata* was calculated based on the dry weight of samples m^{-2} and expressed in kg ha^{-1}. In order to estimate the nutrient content of Singapore daisy, twelve whole plant samples of *Sphagneticola trilobata* in the flowering stage were randomely collected, washed to remove dirt, dried under shade for two weeks and then it was dried in hot air oven at 65 +/- 5 °C till it attained same consecutive weights. The dried plant samples were ground and sieved to pass through 0.5 mm sieve. The required samples were weighed out and analysed for nutrients. The content of nitrogen, phosphorus and potassium in the plant samples were determined by Micro Kjeldahl method, Spectrophotometric method and Flame photometry respectively (Jackson, 1973) and expressed as percentage. Calcium and magnesium content in plant samples were determined by Atomic absorption spectrophotometry (Fishman and Erdmann, 1973). Chlorophyll content of the plant sample was determined by the method given by Arnon (1949) using acetone. The plant samples were weighed and macerated with 10 ml of 80% acetone. The contents were centrifuged at 3000 rpm for 10 minutes. The supernatant was extracted and made up with acetone. Absorbance was measured at 480, 510, 645, and 663 nm by using a UV-visible spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll content was expressed as milligram of chlorophyll per gram of leaf tissue (mg g^{-1}).

Chlorophyll a = [(12.7 x OD at 663) - (2.69 x OD at 645)] x V/1000 x W

Chlorophyll b = [(22.9 x OD at 645) – (4.68 x OD at 663)] x V/1000 x W

Total chlorophyll = [(8.02 x OD at 663) + (20.2 x OD at 645)] x V/1000 x W

where, V = volume of acetone used; W = weight in grams

In order to do the analysis of heavy metal, three composite samples of shoot, root and soil from each of the three locations were taken for analysis. Approximately one kg of soil samples from 0-15 cm depth were collected randomly from ten points each from the infested field. The samples were then air dried at room temperature for two weeks, crushed and pulverized to pass through 2 mm sieve and one composite sample from each location was prepared for analysis. The plant samples were collected from the same sites from where the soil was collected. The carefully uprooted plants were thoroughly washed to remove mud and dirt and dried for two weeks. Then the below ground (root) and aerial (shoot) portions were separated and oven dried at 65 +/- 5° C till they attained same consecutive weights.

The weighed samples were digested using the diacid digestion mixture of Nitric acid and perchloric acid (9:4) and the presence of heavy metals were analysed using Atomic Absorption Spectrophotometer (Hesse, 1971) from Central Instruments Laboratory- College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The concentration, transfer and accumulation of heavy metals from soil to roots and shoots was evaluated in terms of Biological Concentration Factor (BCF), Translocation Factor (TP) and Bioaccumulation Coefficient (BAC) using the following equations as suggested by Tukura *et al.* (2012).

BCF = heavy metal content in root/ heavy metal content in soil

BAC = heavy metal content in shoot/ heavy metal content in soil

TF = heavy metal content in shoot/ heavy metal content in root

Like many other weed species, the luxuriant growth of Singapore daisy has resulted in using the plant as a fodder for cattle by the local farmers. Hence analysis of proximate principles was also done to assess the nutritive value of Singapore daisy. The nutritive value of the weed biomass of twelve samples were assessed by the analysis of proximate principles, *i.e*., moisture, total ash, acid insoluble ash, crude protein (CP), crude fibre (CF) and ether extract/crude fat (EE) based on the official methods of analysis (AOAC,2012). Nitrogen Free Extract (NFE) was calculated on dry weight basis by the following formula.

NFE (%) = 100- (CF % + CP %+ EE %+ Ash %)

The presence of antinutritional factors such as saponin and tannin was also evaluated. Saponin content was estimated using the method by Nahapetian and Bassiri (1974). Plant samples were washed, shade dried, oven dried and powdered. 10 grams of sample powder was mixed with 100 ml of 20 % ethanol, heated at 55°C for 5 hours and filtered. This process was repeated with another 100 ml of ethanol and the combined extracts were reduced to $1/5^{th}$ volume at 90°C. The concentrate was extracted twice with 10 ml diethyl ether and the aqueous layer was separated using separating funnel. The extract was washed with NaCl solution, evaporated at 50°C to semi-dried form, and then dried to a constant weight. The saponin content was expressed in mg/kg of the dried sample.

The quantity of tannin in the plant samples were determined by the Folin-Denis method by reducing phosphotungstomolybdic acid in an alkaline solution to produce a blue colour which is measured at a wavelength of 700 nm. The necessary reagents includes Folin-Denis reagent, sodium carbonate solution and tannic acid standards. Tannin extraction involves boiling the sample, centrifuging, and adjusting the volume. The sample is then reacted with the Folin-Denis reagent and sodium carbonate, and the absorbance is read after 30 minutes. A standard graph using tannic acid is prepared for the calculation of tannin content (Schanderl, 1970).

3. RESULTS AND DISCUSSION

3.1 Nutrient content and uptake

The nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) content of Singapore daisy was estimated to be 1.17%, 0.38%, 1.29%, 0.84% and 0.24% respectively. The nutrient uptake values for these elements were recorded as 151.23 kg ha^{-1} for nitrogen, 49.12 kg ha^{-1} for phosphorus, 166.75 kg ha^{-1} for potassium, 108.50 kg ha^{-1} for calcium and 31.02 kg ha^{-1} for magnesium. The order of nutrient concentration in plant was found to be K > N > Ca > P > Mg (Table 1.).

Table 1. Nutrient content and uptake of Singapore daisy

NUTRIENTS	CONTENT (%)	UPTAKE (kg ha^{-1})
Nitrogen	1.17	151.23
Phosphorus	0.38	49.12
Potassium	1.29	166.75
Calcium	0.84	108.5
Magnesium	0.24	31.02

The high biomass production is supported by the plant's chlorophyll content which is essential for photosynthesis. Singapore daisy has a chlorophyll a (2.205 mg/g) and chlorophyll b (1.125 mg/g), with a total chlorophyll of 3.32 mg/g in plant tissue. The chlorophyll content indicate that the plant is very efficient in capturing sunlight and converting it into energy through photosynthesis (Slattery *et al.,* 2017). The efficiency in photosynthesis leads to rapid plant growth, allowing Singapore daisy to take up more nutrients from the soil to support its development.

The nutrient content of Singapore daisy was similar to that of rice straw with nitrogen, phosphorus and potassium content of 0.7%, 0.23 and 1.75 kg/ha respectively. The calcium content was found to be 0.35% and magnesium content was found to be 0.2%, indicating that Singapore daisy had similar nutrient content as rice straw (Goswami *et al.*, 2020) indicating that Singapore daisy has comparable nutrient profile to rice straw. This suggest that Singapore daisy could be used as a viable alternative to rice straw for composting and soil fertility management.

The decaying biomass of Singapore daisy can play a vital role in nutrient cycling, ensuring that there is no net loss of nutrients and also increase the organic matter of soil (Afzal *et al.,* 2023). Higher biomass production (12.92 t ha^{-1}) and nutrient uptake of Singapore daisy, indicate that uncontrolled spread of the plant could lead to depletion of soil nutrient over time. Similarly, in other invasive species, nutrient mining has resulted in the formation of monotypic stands (Nunez and Paritsis, 2018).

3.2 Phytoremediation

The heavy metal content in Singapore daisy-invaded soil from three locations is presented in Table 2, and the heavy metal content in the shoot and root of Singapore daisy from the same locations is shown in Table 3.

Table 2. Content of heavy metals in Singapore daisy invaded soil

Heavy metal	**Content (mg kg^{-1})** ***Kalichampothi***	**Content (mg kg^{-1})** ***Nileshwaram***	**Content (mg kg^{-1})** ***Azhithala***
Arsenic (As)	31.72	38.64	21.72
Lead (Pb)	11.64	5.08	1.68

Cadmium (Cd)	3.36	2.84	2.16
Chromium (Cr)	249.68	69.56	30.76

Table3. Heavy metal content in shoot and root of Singapore daisy

Heavy metals	*Kalichampothi*		*Nileshwaram*		*Azhithala*	
	Shoot (mg kg^{-1})	**Root (mg kg^{-1})**	**Shoot (mg kg^{-1})**	**Root (mg kg^{-1})**	**Shoot (mg kg^{-1})**	**Root (mg kg^{-1})**
Arsenic (As)	-	-	-	138.30	59.80	133.00
Lead (Pb)	6.20	6.00	4.80	6.00	5.80	5.20
Cadmium (Cd)	7.40	6.40	6.40	8.33	7.00	5.60
Chromium (Cr)	51.80	38.40	31.00	52.00	42.40	54.80

Heavy metal contamination is a widespread threat that degrades soil, water, plant health and ecosystem. Phytoremediation is a cost-effective and ecologically friendly technique that uses plants to immobilise, absorb, reduce toxicity, stabilise, or degrade heavy metals released into the environment by various sources (Kafle *et al*., 2022). Plants such as hybrid poplar, willows, sunflowers, alpine pennycress, clover, Indian mustard, redroot pigweed, and ferns are commonly used for commercial phytoremediation. *Jatropha curcas* has the potential of phytoremediation of Fe, Al, Cu, Mn, Cr, Zn, As and Hg (Meena et al., 2019). Phytoremediation techniques for heavy metal-contaminated soils include phytostabilization, which reduces heavy metal bioavailability in soil and phytoextraction, which removes heavy metals from soil. Phytostabilization involves using metal-tolerant plant species to immobilise heavy metals belowground and reduce their bioavailability. This prevents metals from entering the ecosystem and food chain (Wong, 2003).

Heavy metals can be stabilised by precipitation, reduction in metal valence in the rhizosphere, absorption in root tissues or adsorption on root cell walls (Ginn *et al*., 2008). A plant is considered suitable for phytostabilization if its biological concentration factor (BCF) value is more than 1 and translocation factor (TF) value is less than 1 (Mkumbo *et al.*, 2012; Radziemska *et al*, 2017) as shown in Table 4. In case of cadmium, chromium, arsenic and lead, it was found that the BCF value was found to be more than 1 and TF was less than 1. Therefore, Singapore daisy has a potential for phytostabilisation. These plants stabilise heavy metals belowground and reduces their leaching into groundwater. It also limits wind dispersal of heavy metal-containing soil particles (Mench *et al.,* 2010). Singapore daisy can therefore be considered as a potential choice for phytostabilization of arsenic, cadmium, lead and chromium.

Table 4. BAC, BCF and TF of Singapore daisy

Heavy metals	*Kalichampothi*			*Nileshwaram*			*Azhithala*		
	BAC	**BCF**	**TF**	**BAC**	**BCF**	**TF**	**BAC**	**BCF**	**TF**
Arsenic (As)	-	-	-	-	3.57	-	2.75	6.12	0.44
Lead (Pb)	0.53	0.51	1.03	0.94	1.18	0.8	3.45	3.09	1.11
Cadmium (Cd)	2.20	1.90	1.15	2.25	2.93	0.76	3.24	2.59	1.25
Chromium (Cr)	0.20	0.15	1.34	0.44	0.74	0.59	1.37	1.78	0.77

In phytoextraction, plants take up heavy metals from soil, translocate and accumulate in the above ground part of the plant such as stem and leaves (Salt *et al*., 1995). This method is an effective way to remove toxic heavy metals from soil. A plant is considered suitable to phytoextraction if its biological accumulation coefficient (BAC) value and translocation factor (TF) value are more than 1 (Lorestani *et al*., 2013; Hussain *et al*, 2022). BAC refers to the ratio of heavy metal content in shoot to heavy metal content in soil, while TF is the ratio of heavy metal in shoot to that of root (Tukura *et al*., 2012). High root-to-shoot metal translocation suggested that these plants had crucial qualities to be exploited in phyto-extraction of these metals, as indicated by Lazaro *et al*. (2006).

These values indicate how well a plant can absorb and translocate them to the above ground plant parts. In the case of cadmium and lead, the BAC and TF was found to be more than 1 indicating the potential of Singapore daisy to be used for phytoextraction of cadmium and lead. Therefore, Singapore daisy is a viable candidate for removing heavy metals from soil.

The plant used for phytoextraction should possess certain traits such as fast growth, high biomass production and good adaptability to various environmental condition (Seth, 2012). Singapore daisy meets these criteria, making it suitable for phytoremediation. The fast-growing nature of Singapore daisy allows it to establish quickly and cover large areas accelerating heavy metal uptake. The plant is also adaptable to thrive under different soil types and stress conditions (Azeem *et al*., 2020).

The aboveground biomass is another key factor that determine the phytoextraction potential of a plant species. Plants with high above ground biomass can accumulate heavy metal on the above ground portion of the plant to a great extent (Yan *et al.,* 2020). The average dry biomass production of Singapore daisy was 12.92 tonnes/ha and can accumulate large quantity of heavy metal. Singapore daisy with its fast growing nature, adaptability and high biomass production and its phytoremediation potential makes it a versatile tool for soil remediation.

3.2 Fodder

Singapore daisy is utilised as a forage material by some farmers in local regions because of its luxuriant growth and availability throughout the year. Therefore, the nutritive value of biomass was assessed by analysing the proximate principles based on the official methods of analysis (AOAC, 2012). Moisture content, total ash, acid insoluble ash, crude fibre, crude protein, crude fat/ether extract and nitrogen free extract were determined on dry weight basis (Table 5).

Table 5. Proximate principles of Singapore daisy

Parameters	Content (%)
Moisture	76.64
Total ash	11.18
Acid insoluble ash	2.38
Crude protein (CP)	7.34
Crude fibre (CF)	11.59
Ether extract (Crude Fat)	2.96
Nitrogen free extract (NFE)	66.93
K/(Ca+Mg)	1.19

Proximate analysis enables legitimate comparisons of feeds based on specific nutrients, allowing one to determine how much superior one feed is to another in terms for particular nutrients (Galyean, 1980). The crude protein content of Singapore daisy was estimated as 7.34%, which was similar to the crude protein content of Setaria grass (*Setaria anceps*), typically ranging between 4.8 to 18.4% and Para grass (*Brachiaria mutica*), ranging from 2.8 to 16.1%. It is also higher than the crude protein content of Gamba grass (*Andropogon gayanus*), which is 5.5% (KAU, 2024).

The crude fibre content of Singapore daisy was estimated as 11.59 % which was lower than the crude fibre content of fodder cowpea (20%) (KAU, 2024). According to Fernandez and Jorgensen (1986), lower crude fibre content suggests easy digestibility therefore Singapore daisy is more digestible than fodder cowpea. The nitrogen free extract of Singapore daisy was 66.93% which can be compared to the NFE of Napier grass, which was 51.88 (Kamaruddin *et al.*, 2020). According to Rivera and Parish (2010), crude protein content ranging from 7 to 8 % and moisture content of 71 to 74 % is a standard set for good quality silage and hence Singapore daisy could be considered as a potential choice for silage production.

According to Kumar and Soni (2014), if the K/(Ca+Mg) ratio of forage grasses is above 2.20, it may cause a metabolic disease in cattle known as grass tetany, caused due to magnesium deficiency. In Singapore daisy, the ratio was 1.19, which was well within the safe limit.

The presence of saponin and tannin was detected in Singapore daisy. According to Fayique and Thomas (2018), there are nine major secondary metabolites like saponin, tannins, nitrates, mimosine, cyanogenic glycosides, oxalates, phytates, alkaloids and protease inhibitor; the toxic effect of which can be minimised by variety of methods, so as to increase the fodder quality. Chanchay *et al*., (2009) observed that when plants are dried at 60 ^{o} C for 24 hours, there was 80% reduction in tannin content. It was also found that there was 99% reduction in tannin content when the dried leaves were soaked in water for seventy two hours, followed by drying at 60^{o} C for 48 hours. Tannin rich fodder when combined with concentrated ration can be fed to animals (Ramteke *et al*., 2019). Saponins, characterised by their bitter taste and foaming properties can impact animal performance and metabolism by reducing erythrocyte haemolysis, lowering blood and liver cholesterol, reducing growth rate, causing bloating in ruminants, inhibiting smooth muscle activity, inhibiting enzymes and decreasing nutrient absorption. Washing the feed with water helps reduce the bitterness caused by saponins, making it more palatable (Kumar *et al*., 2017).

4. CONCLUSION

Singapore daisy has the potential to be used in phytoremediation of contaminated soil, supported by its substantial average biomass production of 12.92 tonnes ha^{-1}. The values of proximate principles of Singapore daisy revealed that it could serve as a valuable fodder crop as the nutritive values were comparable with common fodder crops of Kerala. Additionally, the K/(Ca+Mg) ratio of 1.19, which is regarded safe for animal feed. Even though there are effective techniques for removing the anti-nutritional factors for using as cattle feed, the phytoremediation potential of the plant in contaminated soils indicate there is requirement for further studies to justify its use as fodder.

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