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Tecoma stans Essential Oils: A Useful Additive in Food Industries

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Abstract: Essential oils (EOs) and their constituents have been the focus of several researches because of their multiple biological activities. Among these activities are antioxidant and antimicrobial, which make EOs important material in many industries. Food industries now use EOs as food additives and also incorporate them in food packages to increase the food shelf life by preventing food spoilage caused by oxidation and microbial attacks. There are many plants bearing essential oils that are yet to be investigated. Therefore, in this study, the EOs hydrodistilled from the leaves, stem, seeds and flowers of *Tecoma stans* were examined for their antioxidant and antimicrobial activities using DPPH radical-scavenging assay and Agar diffusion method respectively. Data were analyzed using Graph Pad Prism software. The seed (IC_{50} = 33.03 µg/ mL) and stem (IC_{50} = 6.44 µg/ mL) EOs exhibited higher radical-scavenging potential than the EOs from the leaves (168.62 µg/ mL) and flowers (104.94 µg/ mL) as well as that of reference compound, α-tocopherol (IC_{50} = 81.58 µg/ mL). There was no significant activity against the bacterial but moderate inhibitions of the fungi were observed (Zones of Inhibition, 1.8-8.1 mm). Their antimicrobial activity was lower than those of the standard drugs (gentamycin, 9.0-11.5 mm; ketoconazole, 10.3-21.0 mm). No significant difference between the activity of each of the EOs against the tested organisms ($P > 0.05$). This study showed that *Tecoma stans* seeds and stems EOs possess antioxidant properties which could make them useful in the food industry as food additives and food packaging materials.

Keywords: Antioxidant, Antimicrobial, Food Additives, Food Packages

1. Introduction

Food spoilage is a condition at which a food product becomes unsuitable to ingest by the consumer. It is majorly caused by many external factors like heat, water, ultraviolet light, pressure and microorganisms from either unhygienic production, poor storage conditions or mode of transportation [1]. One of the preventive method adopted by food industry is the use of natural antioxidants and antimicrobial agents from plants which are more safe than the synthetic ones when trying to avoid the problem of toxicity [2]. Many fungi and bacteria contribute up to 50 % of all food spoilage [3]. Common examples are *Aspergillus* and yeast species for fungi and *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* for bacteria. Essential oils among plants extracts have shown significant antioxidant activity and antimicrobial property against wide range of microorganisms. Besides, they have multibiological activities which make them useful in many industries such as pharmaceutical, cosmetic, food as well as medicine [4]. Many food industries now use essential oils as natural flavoring agents, natural preservatives and in food packaging material to enhance their product's shelf life [5].

Tecoma stans (L.) Kunth of Bignoniaceae family, commonly known as the yellow trumpet tree and locally called Awun in Nigeria by yoruba tribe is a flowering plant, which originated from neotropical America and introduced to other regions like Hawaii, India, Philippines and Africa [6]. *Tecoma* and many species of Bignoniaceae have either commercial or ethnobotanical uses, although most common among them are those planted as ornamentals. The leaf, root, stem and flowers of *Tecoma stans* are all used in traditional medicine in the treatment of several human ailments like gastro-intestinal disorder, diabetes, cancer, heart disease and syphilis [7]. The

plant also provides wood for construction purpose, firewood, charcoal and also used in the synthesis of nanoparticles [8]. Wound healing, anti-inflammatory, mosquitocidal, antidiabetic, antibacterial, antifungal, antioxidant, hypoglycemic and antitumor properties of both aqueous and organic extracts from different parts of the plant have been examined [7, 9-11]. Despite a lot of reports on the antioxidant and antimicrobial activities of the plant's extracts, there is dearth of information on the biological activities of the essential oils from the plant. Therefore, this study was carried out to evaluate the antioxidant and antimicrobial properties of essential oils extracted from the leaves, stem, seeds and flowers of the plant.

2. Methodology

2.1. Plant Collection and Identification

Tecoma stans was collected at Saunders road, University of Ibadan, Oyo State, Nigeria (7° 23' 0.2" N/3° 54' 28.8" E). The plant was identified at the herbarium in the Botany Department, University of Ibadan and authenticated at the Forest Research Institute of Nigeria where a voucher specimen was deposited (FHI 112524).

2.2. Essential Oil Extraction

The essential oils were hydrodistilled from the plant accordance to the British Pharmacopeia (1980) specification [12] with some modifications. Plant parts (leaf, stem, seed, flower) were air-dried for three weeks and pulverized. The pulverized plant materials (500 g) were covered with water inside a round-bottom flask which was placed on heating mantle, a source of heat. When the water started boiling, the oils distilled from the specialized cells where they were stored in the plant parts. Both the oil vapour and the water vapour passed through the Clevenger-type apparatus that was connected to the mouth of the round bottom flask to its receiver arm which contains analytical grade *n*-hexane and water. The hexane helps to trap the essential oils. After 3 h of heating, the oils were collected and separated from moisture by passing the mixture over sodium sulphate (Na_2SO_4). The oils were stored in sealed glass vials, labeled accordingly and placed inside the refrigerator at 4°C prior to bioassay.

2.3. Antioxidant Assay

The antioxidant activity of the essential oils was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay which measures the ability of the essential oils to donate proton or electron to free-radicals. The assay was carried out based on the procedure highlighted by Saleh *et al.,* [13] with some modifications. The mixture of 1.5 mL of the essential oil samples dissolved in methanol (5 mg/mL, 25 mg/mL and 100 mg/mL) and 1.5 mL of 0.2 mM DPPH were incubated in the dark at room temperature for 20 minutes. The absorbance at 517 nanometers was recorded as $A_{(sample)}$ via CE 2021, 2000 series double beam ultraviolet-visible spectrophotometer. $A_{(blank)}$ was also recorded using the same procedure but without essential oils. The experiment was performed in triplicates and the percentage inhibition was calculated according to the formula below:

$$\% \text{ inhibition} = A_{(blank)} - A_{(sample)} / A_{(blank)} \times 100$$

The concentration of the oil that gives 50% inhibition (IC_{50}) was determined from the graph of percentage inhibition against concentration of the essential oil using Microsoft EXCEL [14]. The activity of α-tocopherol was likewise evaluated as positive control.

2.4. Antimicrobial Assay

The antibacterial activity of the essential oils was evaluated using the method described by Hood *et al*., [15] with some modifications. The oils were tested against two bacteria standard strains (*Staphylococcus aureus* ATCC 6571 and *Escherichia coli* ATCC 25925) and three clinical fungi isolates (*Candida albicans*, *Aspergillus niger* and *Fusarium solani*) from University of Ibadan College Hospital and the analysis was carried out in Pharmaceutical Microbiology Laboratory, University of Ibadan. Bacteria strains were maintained on Mueller-Hinton Agar while fungi on Sabouraud Dextrose Agar (SDA), and each medium was prepared according to the manufacturer's instructions. Diluted overnight cultures (10^{-2} CFU/ mL) were inoculated each into sterile agar. Seven wells of uniform diameter were created in the seeded agar plates using 8 mm cork borer and labelled. Different concentrations (1 µL/mL, 10 µL/mL, 100 µL/mL) of essential oils, positive control (10 µL/mL gentamycin as antibacterial and 200 µg ketoconazole as antifungal) and negative control (DMSO) were introduced into the wells accordingly and allowed to diffuse into the seeded agar for 30 mins before incubating for 24 h at 37°C and 48 h at 25-32°C for bacteria and fungi, respectively. The assay was performed in duplicate and the activity of the test samples against the microbes were evaluated by measuring the diameter of zones of inhibition of the organisms without the diameter of the wells in millimeters.

2.5. Statistical Analysis

The statistical analysis was performed on the antimicrobial experimental data using Graph Pad Prism software, version 3.0 for windows. P value less than 0.001 ($P < 0.001$) was considered statistically significant while P value greater than 0.05 ($P > 0.05$) was considered non-significant.

3. Results

Table 1. *Extraction result.*

Essential oils	Colour	% Yield
Leaves	Pale yellow	0.62
Seeds	Colourless	0.36
Stem	Colourless	0.32
Flowers	Pale yellow	0.49

Hydrodistillation of the dried leaves, seeds, stem and flowers of *T. stans* gave a varying colour of colourless and

pale yellow and percentage yield range of 0.32% - 0.62 % (Table 1). The chemical constituents of the essential oils had been reported earlier [16]. The major constituents and the classes of compounds identified are presented in Table 2.

Table 2. *Dominant constituents of T. stans essential oils.*

Constituents	l.r.i.	Percentage composition (%)			
		Leaf	Stem	Seed	Flower
1-octen-3-ol	981	24.8	3.7	5.5	7.9
3-octanol	994	6.0	7.0	6.0	-
Linalool	1101	7.7	11.4	7.4	10.8
α-terpineol	1191	0.5	5.0	11.7	1.3
β-selinene	1487	-	5.4	9.3	-
(E)-β-ionone	1487	2.7	-	-	11.2
2,6,10-trimethylpentadecane	1642	10.4	10.7	-	8.4
β-eudesmol	1650	-	5.9	-	-
Pentadecanal	1716	8.9	-	-	3.1
Total monoterpenes identified		-	0.7	4.3	-
Total monoterpenoids identified		16.1	17.3	26.3	12.1
Total sesquiterpenes identified		-	6.9	13.5	3.7
Total sesquiterpenoids identified		11.8	16.4	5.4	3.7
Percentage identified		91.5	81.4	88.5	95.8

Lri- Linear retention indices from the analyses value.

3.1. Antioxidant Activity

The radical scavenging potential of *T. stans* essential oils increases as the concentration of the oils increases. The antioxidant activity is reported as IC_{50} (Table 3). The value was obtained from the straight line equation generated by plotting the values of percentage inhibitions of the radicals against the concentrations of the volatile oils and α-tocopherol (Figures 1-5). The value of x when y is 50 % in the linear equation gives the IC_{50}.

Table 3. *Antioxidant activity of T. stans essential oils.*

Test sample	Absorbance at 517 nm			IC_{50} (µg/mL)
	5 µg/ mL	25 µg/ mL	100 µg/ mL	
Leaves EO	0.926	0.905	0.898	168.62
Seed EO	1.046	0.918	0.872	33.03
Stem EO	1.045	1.005	0.873	6.44
Flowers EO	1.160	1.028	1.020	104.94
α-Tocopherol	1.447	0.988	0.979	81.21

Absorbance of control (DPPH + Methanol) = 2.003

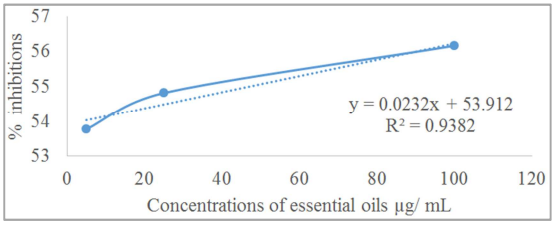

Figure 1. *Antioxidant activity of Tecoma stans leaf essential oil.*

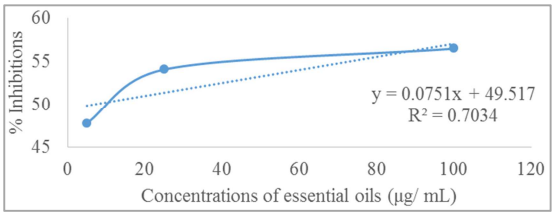

Figure 2. *Antioxidant activity of Tecoma stans stem essential oil.*

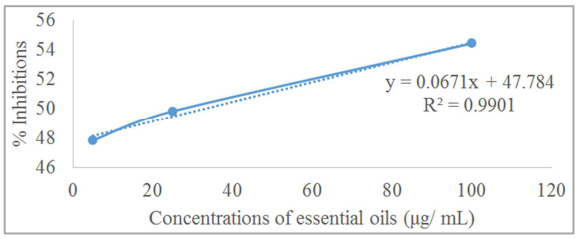

Figure 3. *Antioxidant activity of Tecoma stans seed essential oil.*

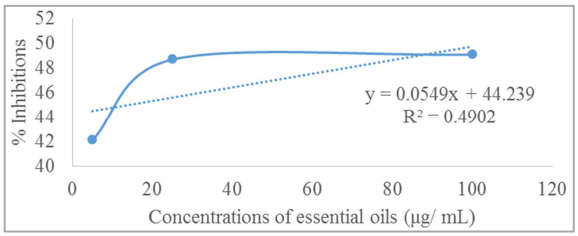

Figure 4. *Antioxidant activity of Tecoma stans flower essential oil.*

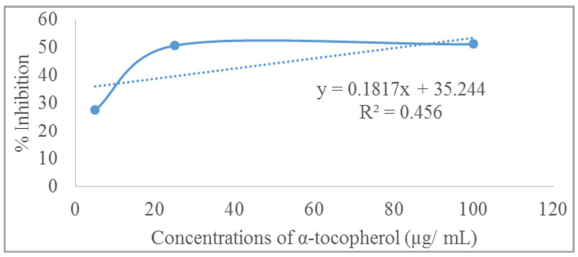

Figure 5. *Antioxidant activity of α-Tocopherol.*

3.2. Antimicrobial Activity

The antimicrobial activities of *Tecoma stans* essential oils are presented in Table 4. The oils have no antibacterial activity except the leaf oil which inhibited both *E. coli* and *S. aureus* at 100 µL/mL, although they were weak inhibition zones (< 2 mm). However, all the oils exhibited moderate antifungal activity when compared with the standard antibiotics.

Table 4. *Antimicrobial activity of T. stans essential oils.*

Essential oils	Conc (µL/mL)	Zones of Inhibition (mm)				
		S. aureus	***E. coli***	***C. albicans***	***F. solani***	***A. niger***
Leaf	1	0.0a	0.0a	0.0a	0.0a	0.0a
	10	0.0a	0.0a	2.3a	2.1a	2.1a
	100	1.3a	1.7a	7.8a	4.1a	6.1a
Stem	1	0.0a	0.0a	0.0a	0.0a	0.0a
	10	0.0a	0.0a	2.2a	1.6a	1.8a
	100	0.0a	0.0a	6.1a	3.9a	4.0a
Seed	1	0.0a	0.0a	2.1a	2.1a	0.0a
	10	0.0a	0.0a	4.0a	3.9a	0.0a
	100	0.0a	0.0a	6.2a	6.0a	1.4c
Flower	1	0.0a	0.0a	3.9a	1.9a	3.9a
	10	0.0a	0.0a	6.0a	3.9a	6.0a
	100	0.0a	0.0a	8.1a	6.1a	8.1a
+ve control		11.5b	9.0b	21.0b	10.3b	10.5b
-ve control		0.0	0.0	0.0	0.0	0.0

Mean value of inhibition zone followed by different letters in the same column indicates there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicates no significant difference between the mean ($p > 0.005$)
+ve control- Gentamycin for antibacterial assay; Ketoconazole for antifungal assay
-ve control- DMSO and n-hexane

4. Discussion

Essential oils of *T. stans* seed and stem gave higher antioxidants activity than the other two parts and the synthetic antioxidant α-tocopherol. The lower the IC_{50} values the higher the scavenging ability. The antioxidant properties of the essential oils are related to their chemical constituents. In the previous accounts, essential oils dominated by monoterpenes or their oxygenated derivatives (containing alcohol, carbonyl -aldehyde or ketone and ester functional groups) demonstrated high antioxidant activities [17, 18]. Furthermore, Bicas *et al.,* [19] reported that presence of oxygenated monoterpenes like α-terpineol and linalool in essential oils could be responsible for high antioxidant activity of the oil. These facts justify the radical-scavenging ability of the stem and seed oils that showed higher antioxidant activity in this study than the other two parts (flowers and leaves). Among the dominant constituents of all the *Tecoma stans* essential oils was linalool, while α-terpineol was found at higher percentage in the stem and seed oils. Likewise, monoterpene hydrocarbons were found only in the stem and seed oils as well as greater percentage of oxygenated monoterpenes compared with the leaf and flower oils [16]. These could have contributed to the higher radical-scavenging activity of the stem and seed oils. In addition, synergistic effects of the other constituents of the oils could also have contributed to their activity. The flowers and leaves essential oils also showed moderate activity but lower than the activity of the synthetic antioxidants.

Likewise, good antimicrobial activities of essential oils were credited to the presence of high percentage of monoterpenes, sesquiterpenes and their related phenols, alcohols as well as other oxygenated compounds present due to their mode of inhibiting the microorganisms' growth [20]. Although these did not reflect in the activity of *T. stans* essential oils. All the oils contain an appreciable quantity of oxygenated compounds (monoterpenes and sesquiterpenes) and yet not as active as the reference antibiotics. These could be attributed to antagonistic effects due to the interaction between non-oxygenated and oxygenated monoterpene hydrocarbons [21]. Statistical analysis of the antimicrobial activity of the essential oils showed that there was no significant difference in their activity against each of the tested organisms ($P > 0.05$). On the other hand, there were significant differences between the activity of the positive control and each of the oils ($P < 0.001$).

There is no report on the antimicrobial and antioxidant activities of *T. stans* essential oil but the activities of various aqueous and organic extracts from different parts of the plant had been reported [7, 9, 22-27]. The extracts exhibited moderate to high antimicrobial activity against wide range of human pathogens including the organisms used in this research work as well as high antioxidant property.

5. Conclusion

The present study showed that essential oils extracted from *Tecoma stans* stem and seeds possess good antioxidant properties, which could find application in the food industry as natural food additives and also incorporated in to the food packaging material to improve the food shelf life in order to prevent food spoilage by oxidation. Further study should be conducted on the antioxidant and antimicrobial potential of the essential oils in food models.

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