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DPPH Radical Scavenging Activities Of The Air-dried Leaves Essential Oil From *Taminalia catappa* LINNAEUS and *Alternanthera pungens* KUNTH

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ABSTRACT

Essential oils are aromatic, volatile, and lipophilic compounds extracted from various parts of plants, such as flowers, leaves, stems, roots, seeds, bark, and fruits. They are highly valued for their flavor, therapeutic, and aromatic properties, and are widely used in food, medicine, and cosmetic industries. In this study, essential oils were extracted from the leaves of *Terminalia catappa* and Alternanthera pungens using hydrodistillation with an all-glass Clevenger-type apparatus. The oils were then assessed for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with ascorbic acid as the reference standard. The extracted oils appeared as cloudy light yellow to cloudy white liquids with a strong aroma. Yield percentages were 0.25% for *T. catappa* and 0.40% for A. pungens. The inhibition percentages ranged from 84.67% at 25 mg/mL to 54.22% at 100 mg/mL for *T. catappa,* and from 73.87% at 100 mg/mL to 43.90% at 50 mg/mL for A. pungens. The antioxidant evaluation revealed moderate to high radical scavenging activity compared to the standard. Overall, the results highlight the potential of these essential oils as natural antioxidants suitable for applications in food, pharmaceutical, and cosmetic products.

Keywords: Medicinal plant, Essential oil, DPPH, Antioxidant Activity

INTRODUCTION

Essential oils, also known as volatile aromatic oils, are fragrant oily substances extracted from various parts of plants. The extraction process is often labor-intensive and time-consuming, and the method used is crucial, as certain solvent-based techniques may compromise the oils' therapeutic properties. The chemical composition of essential oils varies significantly, with major constituents making up as much as 85% of the oil, while others are found only in trace amounts (Miguel, 2010).

Antioxidants are compounds that neutralize free radicals by donating electrons, transforming them into harmless molecules. A growing body of evidence suggests that cellular damage caused by free radicals contributes to the development of various conditions, including neurological disorders, cancer, cardiovascular diseases, and weakened immune function (Aruoma, 1998; Kamatou and Viljoen, 2010). Oxidative stress, in particular, plays a key role in chronic and degenerative diseases such as cancer, autoimmune conditions, rheumatoid arthritis, cataracts, aging, and neurodegenerative disorders (Willcox et al., 2004; Pham-Huy et al., 2008).

The antioxidant capacity of phenolic compounds is primarily attributed to their redox behavior, enabling them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal ion chelators (Kumar, 2005; Isah, 2021). As a result, naturally occurring antioxidants have gained considerable attention for their potential to protect the human body from oxidative damage and for their ability to safeguard food products against deterioration caused by oxidation (Osawa et al., 1990; Houghton, 1995; Maestri et al., 2006).

Terminalia catappa belongs to the Combretaceae family and is a perennial tree species that thrives in tropical climates, making it widespread across nearly all regions of the country. *Terminalia catappa* is also known by several other names, including tropical almond, wild almond, Indian almond, sea almond, beach almond, and

Malabar almond (Untwal and Kondawar, 2006; Orwa et al., 2009). The plant is refers to as "Afonzon" in Yoruba land, Southwest Nigeria (Gbile, 1984; Burkill, 1985).The juice from the plant is used in the preparation of ointment for scabies, leprosy and other cutaneous diseases (Nair and Chanda, 2008). *Terminalia catappa* has been reported to possess various medicinal properties, including antimicrobial, antidiabetic, antinociceptive, antiparasitic, antifungal, and antibacterial activities (Elizabeth, 2005; Rao and Nammi, 2006; Rajarajan et al., 2010). It also exhibits antioxidant effects (Ko et al., 2002; Mety and Mathad, 2011), anticancer potential (Chu et al., 2007), and is used in the treatment of hepatitis and other liver-related ailments in Taiwan (Lin and Kan, 1990). Additionally, the tree is commonly cultivated in southeastern Nigeria for shade and ornamental purposes (Ezeokonkwo and Dodson, 2004; Agu and Menkiti, 2017).

Various phytochemicals have been identified in extracts from the leaves of *Terminalia catappa,* including gallic acid, triterpenic acids, 4-hydroxyphenylpropionic acid, m-coumaric acid, 3,4-dihydroxybenzoic acid, p-coumaric acid, kaempferol, quercetin, tergallagin, and the glycosides vitexin and rutin (Yun-Lian et al., 2000; Fan et al., 2004; Chyau et al., 2006; Duke, 2008). Additionally, the leaves are rich in flavonoids, tannins, saponins, phytosterols, alkaloids, steroidal glycosides, and phenolic compounds (Punniya and Vijaya, 2014). Citronellyl acetate (64.87%) was the predominant compound in the essential oil of Terminalia bentzoë (Gurib-Fakim and Demarne, 1994), while palmitic acid (35.7%) was the major component in T. chebula (Naik et al., 2010). In Terminalia ivorensis, δ-3-carene (29.4%) and α-pinene (20.9%) were the most abundant constituents (Ogunwande et al., 2019). Additionally, the essential oils extracted from various parts of *T. catappa,* including its fruits, leaves, and nuts, have been found to contain several major phytochemicals, such as α-farnesene (21.3%), hexahydrofarnesyl acetone (12.34%), octadecane (11.7%), hexadecanoic acid (9.5%), dibutyl phthalate (9.1%), 1,2,3-trimethoxy-5-(2-propenyl)-benzene (6.6%), neoisothujol (5.8%), 1,2,4-trimethoxy-5-(1-propenyl)-benzene (4.5%), 6,10,14-trimethyl-2-pentadecanoic acid, 1-(2,3,6-trimethylphenyl)-(E)-3-buten-2-one, geranyl acetone, hexadecanoic acid (21.0%), 2-ethyl-3,6-dimethylpyrazine (19.2%), (Z)-phytol (41.2%), palmitic acid (11.0%), and (E)-nerolidol (4.7%) (Moronkola and Ekundayo, 2000; Wang et al., 2000; Lasekan et al., 2012; Owolabi et al., 2013; Ogunmoye et al., 2020b).

Alternanthera pungens Kunth, a member of the Amaranthaceae family, is commonly known as khaki weed. Locally referred to as "dágunró" in Yoruba-speaking regions of southwestern Nigeria, it is widely distributed across tropical and subtropical areas worldwide (Burkill, 1985). This herbaceous perennial plant typically has prostrate stems, occasionally upright, ranging from 10 to 50 cm in length (Naidu, 2012; Hossain et al., 2018). Livestock generally avoid consuming khaki weed, as it is suspected to be toxic to sheep and pigs, and has been associated with digestive issues and skin problems in cattle (Parsons and Cuthbertson, 2001). Although it is not very palatable to goats, there is no documented evidence of toxicity in them (Simmonds et al., 2000). The plant has demonstrated various pharmacological properties, including spasmogenic, diuretic, anti-HIV, antioxidant, antimicrobial, antibacterial, analgesic, and antidiabetic effects (Petrus and Seetharaman, 2005; Ogundare and Oladejo, 2014). It is also traditionally used to treat gastrointestinal disorders (Adela et al., 2008) and is employed in Mexican folk medicine for managing diarrhea and dysentery (Osuna et al., 2005).

Phytochemical analyses of the plant extracts have revealed the presence of several compounds, including saponins, steroids, alkaloids, triterpenoids, β-spinasterol, glycosides, flavonoids, tannins, phenols, carbohydrates, β-carotene, choline, violaxanthin, zeaxanthin, and lutein (Hossain et al., 2018; Gupta and Saxena, 1987; Mourya et al., 2018).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oil extracted from A. pungens leaves revealed that it is primarily composed of β-ionone (42.18%) and hexahydrofarnesyl acetone (15.53%), with other constituents present in trace amounts (Ogunmoye et al., 2020a). Similarly, the essential oil from the plant's flowers has been found to contain a variety of compounds, including α-pinene (7.40%), β-pinene (6.42%), camphene (4.21%), myrcene (3.61%), p-cymene (4.29%), limonene (3.52%), β-ocimene (2.35%), 1,8-cineole (6.28%), α-thujone (3.62%), α-borneol (4.46%), α-curcumene

(2.36%), camphor (5.52%), linalool (6.29%), geraniol (7.42%), α-terpineol (3.82%), elemol acetate (6.14%), eudesmol (5.38%), azulene (3.16%), along with three unidentified compounds (Gupta and Saxena, 1987).

As a continuation of our investigation into the essential oils of these plants, the present study focuses on evaluating the antioxidant activity of the essential oils extracted from the leaves of *Terminalia catappa* and Alternanthera pungens using the DPPH assay.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Terminalia catappa* and *Alternanthera pungens* were collected from trees located within the premises of Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria. The plant samples were identified and authenticated by Mr. A. O. Adeyemo at the Forestry Research Institute of Nigeria (FRIN), Ibadan, and assigned herbarium numbers FHI-110462 and FHI-110461. Voucher specimens were subsequently deposited at the institute's herbarium. The collected leaves were air-dried at ambient room temperature and then ground into a fine powder for further analysis.

Extraction of essential oils

A total of 200 g of air-dried, pulverized *T. catappa* leaves and 500 g of A. pungens leaves were subjected to hydrodistillation using a Clevenger-type all-glass apparatus for three hours, following standard protocols (British Pharmacopoeia, 1980; Ogundajo et al., 2016). The essential oils obtained were collected in hexane, transferred into sample bottles, and stored in a refrigerator for subsequent analysis.

Estimation of DPPH Radical Scavenging Activity of *Terminalia catappa* and Alternanthera pungens.

Stock solutions of each plant extract were prepared in methanol at a concentration of 10 mg/mL. These were further diluted to obtain concentrations of 100, 50, 25, and 12.5 mg/mL. Ascorbic acid served as the standard, prepared in concentrations ranging from 1 to 100 mg/mL. Each dilution was combined with 1 mL of DPPH solution, and the mixtures were kept in the dark at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a T90+ UV/VIS spectrophotometer, following the method outlined by Saleh et al., 2010, with slight modifications. All experiments were conducted in triplicate.

The percentage inhibition was calculated using the following formula:

$$AA = \frac{Ac - As}{Ac} \times 100$$

Where

A_c = the absorbance of control.

A_s = the absorbance of the test (sample).

AA = the antioxidant activity.

The standard ascorbic acid (vitamin C) was also examined for its antioxidant activity using the same methodology for comparison.

RESULTS

The essential oils extracted from the air-dried leaves varied in appearance, ranging from a cloudy light yellow to a cloudy white liquid, each possessing a strong aroma. The extraction yielded 0.25% for *Terminalia catappa* and 0.40% for *Alternanthera pungens.* Tables 1.0 and 2.0 present the percentage inhibition values of the essential oils from *T. catappa* and *A. pungens,* respectively, compared to a standard reference. Additionally, Figures 1.0 and 2.0 illustrate the percentage inhibition graphs representing the antioxidant activity of the essential oils from *T. catappa* and *A. pungens,* respectively.

DISCUSSION AND CONCLUSION

Based on the data presented in Table 1.0 and Figure 1.0, the essential oil demonstrated varying levels of percentage inhibition across different concentrations ranging from 100 mg/mL to 12.5 mg/mL. Specifically, the inhibition percentages were 54.22% at 100 mg/mL, 82.62% at 50 mg/mL, 84.67% at 25 mg/mL, and 79.07% at 12.5 mg/mL. These results indicate that the antioxidant activity fluctuated with concentration, showing a moderate level of efficacy with inhibition values between 54.22% and 84.67%. The lowest inhibition was recorded at 100 mg/mL (54.22%), while the highest occurred at 25 mg/mL (84.67%). Overall, the essential oil displayed significant DPPH free radical scavenging activity that was generally concentration-dependent, with the exception of the lowest concentration (12.5 mg/mL), which still maintained a high inhibition rate of 79.07%. These findings confirm the antioxidant potential of the oil

as determined by the DPPH assay.

According to the data in Table 2.0 and Figure 2.0, the essential oil exhibited varying levels of percentage inhibition at concentrations ranging from 100 mg/mL to 12.5 mg/mL. The observed inhibition values were 73.87% at 100 mg/mL, 43.90% at 50 mg/mL, 68.13% at 25 mg/mL, and 73.46% at 12.5 mg/mL. These results indicate noticeable variation in antioxidant activity across the tested concentrations. The oil demonstrated moderate antioxidant potential, with inhibition percentages ranging from 43.90% to 73.87%. The lowest activity was recorded at 50 mg/mL (43.90%), while the highest was at 100 mg/mL (73.87%). Unlike the standard, the free radical scavenging activity of this essential oil did not follow a consistent concentration-dependent pattern, as shown by the irregular inhibition values. Nonetheless, the results confirm the presence of antioxidant activity based on the DPPH assay.

The essential oil extracted from *Terminalia catappa* exhibited strong antioxidant activity, with inhibition values ranging from 54.22% to 84.67%, the highest being 84.67% at a concentration of 25 mg/mL. In comparison, *Alternanthera pungens* showed moderate antioxidant activity, achieving a maximum inhibition of 73.87% at 100 mg/mL. In this study, the observed reduction in DPPH color from purple to yellow across various concentrations indicates the presence of electron-donating compounds within the oils, which are capable of neutralizing free radicals by converting them into more stable molecules. These findings confirm the antioxidant potential of the leaf essential oils, aligning with earlier studies on *T. catappa* leaf extracts (Punniya and Vijaya, 2014; Rajesh *et al.,* 2015).

In conclusion, the essential oils demonstrated moderate to strong antioxidant activity in comparison to the radical scavenging potential of the ascorbic acid standard. Notably, the oils extracted from *Terminalia catappa* and *Alternanthera pungens* appear to be promising and rich sources of natural antioxidants. This indicates their potential use as antioxidant agents in industries such as food, pharmaceuticals, and cosmetics. However, further antioxidant assays are recommended to confirm and support the results obtained through the DPPH method.

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Table 1.0: % Inhibition of *T. catappa* leaves essential oil and known antioxidant on DDPH

Concentration (mg/mL)	Oil sample	Ascorbic acid
100 mL	54.22%	91.75%
50 mL	82.62%	89.19%
25 mL	84.67%	87.27%
12.5 mL	79.07%	86.18%

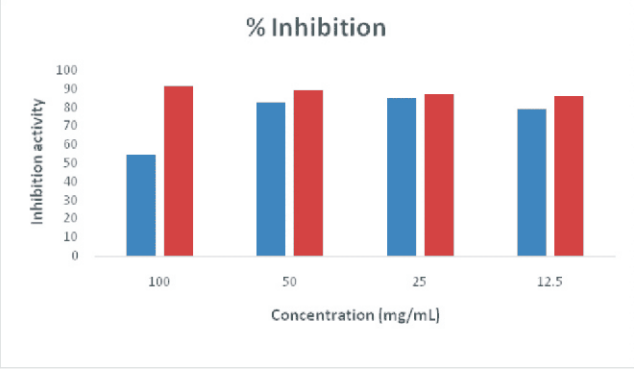

Fig. 1.0: Graph showing the antioxidant activity of the essential oil of *T. catappa*

Table 2.0: % Inhibition of *A. pungens* leaves essential oil and known antioxidant on DDPH

Concentration (mg/mL)	Oil sample	Ascorbic acid
100 mL	73.87%	91.5%
50 mL	43.90%	89.19%
25 mL	68.13%	87.27%
12.5 mL	73.46%	74.41%

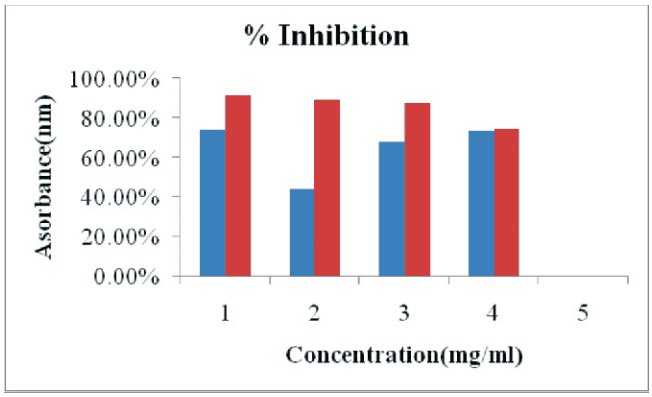

Fig. 2.0: Graph showing the antioxidant activity of the essential oil of *A. pungens*

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