DPPH RADICAL SCAVENGING ACTIVITIES OF THE AIR-DRIED LEAVES ESSENTIAL OIL FROM *Taminalia catappa* LINNAEUS and *Alternanthera pungens* KUNTH

Abstract

Essential oils, a blend of volatile lipophilic compounds derived from various plant parts, including flowers, leaves, stems, roots, seeds, barks and fruits are sought after for their flavours and therapeutic or aromatic properties. They find application in diverse range of products, including foods, medicines, and cosmetics. The essential oils from the leaves of *Taminalia catappa* and *Alternanthera pungens* were extracted through the hydrodistillation method, employing an all-glass Clevenger-type apparatus. The extracted essential oils underwent evaluation for potential antioxidant activity, utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH), with ascorbic acid serving as the standard. The resultant essential oil from the air-dried leaves manifested as cloudy light yellow to cloudy white liquid, exuding a robust fragrance. The percentage yields of essential oils were recorded as 0.25% for *Taminalia catappa* and 0.40% for *Alternanthera pungens.* The % inhibition ratio demonstrated a range from 84.67% (25 mg/mL) to 54.22% (100 mg/mL) for *Taminalia catappa* and 73.87% (100 mg/mL) - 43.90% (50 mg/mL) for (0.25%) *Alternanthera pungens*. Antioxidant assessment unveiled moderate to high radical scavenging activities in comparison to the standard. In conclusion, essential oils from these plants exhibit promising potential as natural antioxidant, making them suitable for incorporation into foods, pharmaceuticals and cosmetics.

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

Introduction

Essential oils, also called volatile odoriferous oil, are aromatic oily liquids extracted from different parts of plants. Extraction of essential oils is one of the most time- and effort-consuming processes. The way in which oils are extracted from plants is important because some processes use solvents that can destroy the therapeutic properties. The proportions of the components present in essential oils vary greatly. Major components can constitute up to 85% of the essential oils, while the remaining components can be present in only trace amounts (Miguel, 2010). Antioxidants are chemical substances that donate an electron to the free radical and convert it to harmless molecule. Increasing evidence has suggested that diseases such as brain dysfunction,

cancer, heart disease and immune system decline may result from cellular damage caused by free radicals (Aruoma OI. (1998; Kamatou and Viljoen, 2010). Also, oxidative stress is a major key player in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases (Willcox *et al.,* 2004; Pham-Huy *et al.,* 2008). The free radical scavenging capability of phenolic compounds is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, singlet oxygen quenchers as well as metal chelators (Kumar 2005; Isah, 2021). Therefore, antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals and also preserve foods from the toxic effects of oxidants (Osawa *et al.,* 1990; Houghton, 1995; Maestri *et al.,* 2006)

Terminalia catappa is a member of the Combretaceae family which is a perennial tree species found in almost all the regions of the Country as it thrives well in the tropics. It is commonly called tropical almond, wild almond, Indian almond, sea almond, beach almond and Malabar almond (Untwal and Kondawar, 2006; Orwa *et al.,* 2009). The juice of *Terminalia catappa* is used in the preparation of ointment for scabies, leprosy and other cutaneous diseases (Nair and Chanda, 2008). Some of the reported medicinal activities includes; antimicrobial, antidiabetics, actinociceptive, antiparasitic, antifungal antibacterial (Elizabeth, 2005; Rao and Nammi, 2006; Rajarajan *et al.,* 2010), antioxidant activities (Ko *et al.,* 2002; Mety and Mathad, 2011), anticancer properties (Chu *et al.,* 2007), hepatitis and liver-related diseases in Taiwan (Lin and Kan, 1990). The plants are also planted for provision of shade and ornamental purposes in the south-eastern Nigeria (Ezeokonkwo and Dodson, 2004; Agu and Menkiti, 2017).

Some of the phytochemicals identified from *T. catappa* leaves extracts includes; gallic acid, triterpenic acids, 4-hydroxyphenylpropionic acid, m-coumaric acid, 3,4-dihydroxybenzoic acid, p-coumaric acid kaempferol, quercetin, tergallagin, glycosides vitexin and rutin (Yun-Lian *et al.,* 2000; Fan *et al.,* 2004; Chyau *et al.,* 2006; Duke, 2008). The leaves also contain flavonoids, tannins, saponins, phytosterols, alkaloids, steroidal glycosides and phenols (Punniya and Vijaya, 2014).

Citronellyl acetate (64.87%) dominated essential oils of *Terminalia bentzoë* (Gurib-Fakim and Demarne, 1994), palmitic acid (35.7%), dominated. *T. chebula* (Naik *et al.,* 2010), δ-3-carene (29.4%) and α-pinene (20.9%) dominated *Terminalia ivorensis* (Ogunwande *et al.,* 2019).

Furthermore, the essential oil of the different parts (fruits, leaves, nuts) of *T. catappa* has been analyzed with some of the following major phytochemicals identified; α-farnesene (21.3%), Hexahydrofarnesyl acetone (12.34%), octadedecane (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, 1-(2,3,6-trimethyl phenyl)-(E)-3-buten-2-one, geranyl acetone, Hexadecanoic acid (21.0%) and 2-ethyl-3,6-dimtheylpyrazine (19.2%), (*Z*)-phytol (41.2%), fatty acid palmitic acid (11.0%), and the (*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 belongs to the Amaranthaceae family and it is commonly known as khaki weed. The plant refers to as ‘dágunró in Yoruba land, Southwest Nigeria, grows widely in tropical and subtropical regions throughout the world (Burkill, 1985). It is an herbaceous perennial plant with stems prostrate, rarely rising and about 10-50 cm long (Naidu, 2012; Hossain *et al.,* 2018). Animals do not generally eat Khaki Weed because it is suspected of poisoning sheep and pigs, and causing digestive disturbances and skin ailments in cattle (Parsons and Cuthbertson, 2001). Khaki Weed is of low palatability to goats, but has no known risk of toxicity (Simmonds *et al.,* 2000). This plant has been shown to exhibit spasmogenic, diuretic, anti-HIV, antioxidant, antimicrobial, antibacterial, analgesic effect and antidiabetic properties (Petrus and Seetharaman, 2005; Ogundare and Oladejo, 2014). The plant is also used as medication for gastrointestinal diseases (Adela *et al.,* 2008), and in traditional Mexican medicine for the treatment of diarrhea and dysentery (Osuna *et al.,* 2005).

Previously reported phytochemicals from the plant extracts are; saponins, steroids, alkaloids, triterpenoids, *β*-spinasterol, glycosides, flavonoids, tannins, phenols, carbohydrate, *β*-carotene, choline, violaxanthin, zeaxanthin and lutein (Hossain *et al.,* 2018; Gupta and Saxena, 1987; Mourya *et al.,* 2018).

The essential oil from GC-MS analysis of *A. pungens* leaves has been reported to be dominated by *β*-ionone (42.18%) and hexahydrofarnesyl acetone (15.53%) with others in trace amounts (Ogunmoye *et al.,* 2020a). Similarly, the flower oil has been reported to contain *α*-pinene (7.40%), *β*-pinene (6.42%), camphene (4.21%), myrecene (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%) and three unidentified compounds (Gupta and Saxena, 1987).

However, in continuation of our research on the essential oil of the plants. This work is therefore focused on the antioxidant activity of the essential oil from the leaves of *Taminalia catappa* and *Alternanthera pungens* using DPPH.

Materials and Methods

Plant materials

Fresh leaves of *Taminalia catappa* and *Alternanthera pungens* were obtained from tree samples within Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria. The samples were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan by Mr. A. O. Adeyemo with herbarium number FHI-110462 and 110461. Voucher specimens of each were deposited at the herbarium in the Institute. The leaves were air dried at room temperature and pulverized before further study.

Extraction of essential oils

The air-dried, pulverized leaves of *T. catappa* (200 g) and *A. pungens* (500 g) were subjected to hydrodistillation in a Clevenger-type all glass apparatus for 3 h in accordance with established procedure (British Pharmacopoeia, 1980; Ogundajo *et al.*, 2016). The oils collected in hexane were preserved in a sample bottle and stored in a refrigerator for further analysis.

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

The stock solution of each plant was prepared in methanol to achieve a concentration of 10 mg/mL. Dilutions were made to obtain concentrations of 100, 50, 25, 12.5 mg/mL. Ascorbic acid was used as a standard in 1-100 mg/mL concentration. The dilutions each were mixed with 1mL of DPPH. After 30 minutes in darkness at room temperature, the samples were analyzed using the T90+UV/VIS spectrometer. The absorbance was measured at 517 nm according to the procedure described by Saleh *et al.,* 2010 with slight modification. The tests were performed in triplicate.

The percentage inhibition was calculated using the following formula:

$$AA = \frac{A_c - A_s}{A_c} \; x \; 100$$

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 extracted essential oil from the air-dried leaves brought about a different level of cloudy light yellow liquid to cloudy white liquid with a strong odour. The percentage yields of the essential oils are *Taminalia catappa* (0.25%) and *Alternanthera pungens* (0.40%).

Discussion and Conclusion

From the results in Table 1.0 and Fig. 1.0, the oil prepared at various concentration from 100 mg/mL to 12.5 mg/mL shows different percentage inhibition. At 100 mg/mL (54.22%), 50 mg/mL (82.62%), 25 mg/mL (84.67%) and 12.5 mg/mL (79.07%) respectively. As the concentration decreases, there is a variance in the percentage inhibitions. The essential oil showed fairly good antioxidant activity having inhibition ratio ranging from 84.67% - 54.22%. The lowest inhibition is 54.22% which was observed at concentration 100 mg/mL while the highest inhibition was 84.67%, observed at concentration of 25 mg/mL. The results of this experiment exhibited marked DPPH free radical scavenging activity in a concentration dependent manner except for the least concentration of 12.5 mg/mL (79.07%). The results obtained by DDPH method showed the existence of an antioxidant activity.

From the results in Table 2.0 and Fig. 2.0, the oil prepared at various concentration from 100 mg/mL to 12.5 mg/mL shows different percentage inhibition. The % inhibitions are; 100 mg/mL (73.87%), 50 mg/mL (43.90%), 25 mg/mL, (68.13%) and 12.5 mg/mL (73.46%). As the concentration decreases, there is a variance in the percentage inhibitions. The essential oil showed fairly good antioxidant activity having inhibition ratio ranging from 73.87% - 43.90%. The lowest inhibition is 43.90% which was observed at concentration 50 mg/mL while the

highest inhibition is 73.87% which was observed at concentration 100 mg/mL. The results of this experiment exhibited marked DPPH free radical scavenging activity which indicated that they are not concentration dependent unlike the standard.

Essential oil of *Taminalia catappa* showed good antioxidant activity having inhibition ratio ranging from 84.67% - 54.22% with the highest inhibition of 84.67% at 25 mg/mL while *Alternanthera pungens* also showed fairly moderate antioxidant activity with highest inhibition of 73.87% (100 mg/mL). In the present study, the percentage reducing power of the essential oils and the change in color of DDPH solution at various concentrations from purple to yellow is an indication that some components of the oils were electron donors that could react with the free radicals and convert them into more stable products. The present results demonstrated good antioxidant activity of the leave essential oil which is in agreement with previously reported studies from *T. catappa* leave extracts (Punniya and Vijaya, 2014; Rajesh *et al.*, 2015)

In conclusion, the essential oils exhibited moderate to high antioxidant activities, when compared to the radical scavenging capability of the ascorbic acid standard. Notably, the essential oils derived from *Taminalia catappa* and *Alternanthera pungens* emerged as potential abundant sources of antioxidants, potentially surpassing the efficacy of the standard ascorbic acid when subjected to further purification. This suggests that these essential oils could serve as natural antioxidants in various applications such as food, pharmaceuticals, and cosmetics. It is advisable to employ additional antioxidant assays to validate the findings 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%

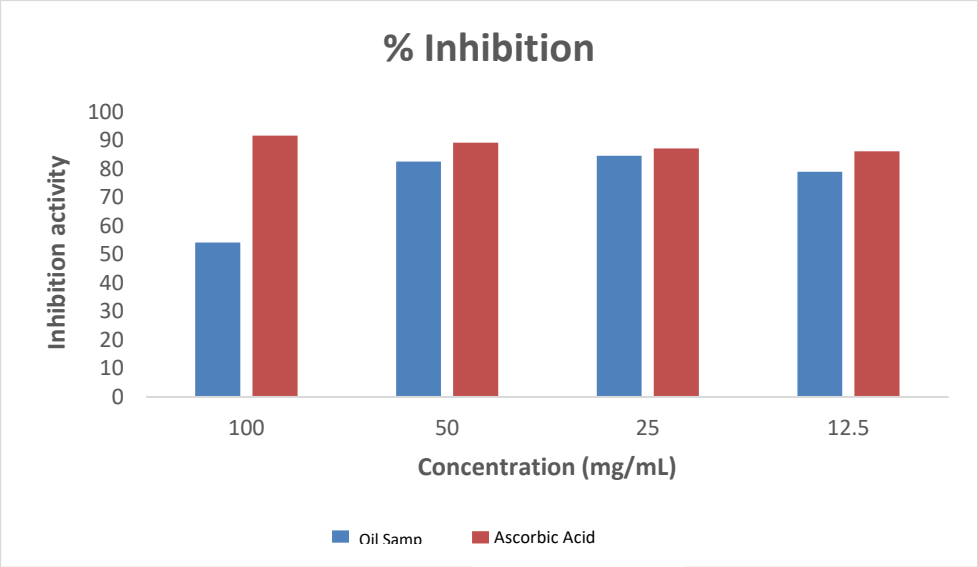

Fig. 1.0: Graph showing the antioxidant activity of the essential oil of *T. catappa*

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%

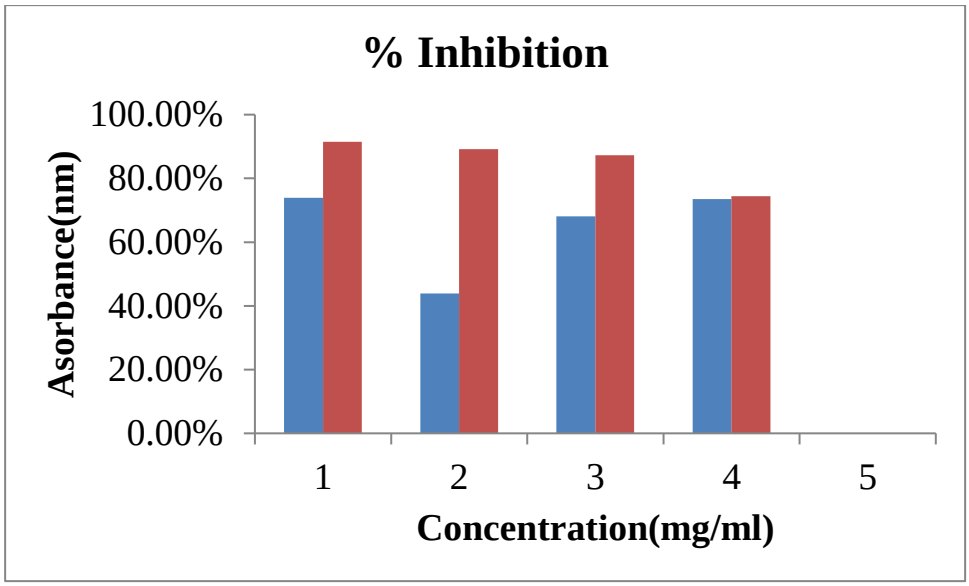

Fig. 2.0: Graph showing the antioxidant activity of the essential oil of *A. pungens*

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