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PRELIMINARY PHYTOCHEMICAL ANALYSES OF TWO VARIETIES OF *ADENIA LOBATA* (Jacq) AND THE ANTIOXIDANT ACTIVITY OF THEIR VARIOUS SOLVENT FRACTIONS

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ABSTRACT
Adenia lobata is a medicinal plant that is traditionally used for the treatment of various diseases such as cancer in some places in eastern Nigeria. Comparative phytochemical analyses were carried out on two varieties of Adenia lobata; Adenia lobata with cordate leaves (ALC) and Adenia lobata with palmate leaves (ALP). ALC was found to possess more phytochemical constituents than ALP. The antioxidant activity of the solvent fractions viz. hexane, dichloromethane (DCM), ethylacetate, butanol and aqueous fractions of each variety of the Adenia lobata was also determined. The highest antioxidant activity (63.87%) was found in the ethylacetate fraction of ALC. These results suggest that the therapeutic use of this plant in the treatment of cancer could be due to its antioxidant activity.
Keywords: Phytochemicals, Adenia, lobata, Medicinal plant, Antioxidant, Anticancer

INTRODUCTION

Plants have been used as medicine for the treatment of diseases for several thousands of years (Abu - Rabia, 2005). During the last few decades there had been an increased interest in the study of these medicinal plants and their traditional uses in different parts of the world (Lev, 2006). According to the World Health Organization, a large percentage of the world's people depend on traditional medicine for their primary healthcare needs (WHO, 1991).

There are considerable benefits in the development of indigenous medicines and in the use of medicinal plants for treatment of various diseases (Azaizeh *et al*., 2003). Traditional knowledge of medicinal plants and their uses by indigenous cultures are not only useful for conservation of cultural traditions, but also for community healthcare and drug development in the present and future (Pei, 2001). Currently, medicinal plants are used as precursors for the synthesis of some useful drugs. In developed countries, about twenty five percent of drugs are derived from plants' bioactive agents (Diallo *et al*., 1999). These plants' bioactive agents, which are also known as secondary metabolites include alkaloids, tannins and flavonoids. They have been reported to have therapeutic activities such as antioxidant, antimicrobial, antidiabetic and antidiarrhea activities (Newman and Cragg, 2007; Ogbulie *et al*., 2007; Asl and Hosseinzadeh, 2008).

Adenia lobata is a medicinal plant that is widely used as traditional medicine in several countries in Africa, including Nigeria (Akendengue *et al*., 2005). It is used for the treatment of cough, respiratory disorder, syphilis, gonorrhea and cancer of the nose (Gill, 1992; Osuagwu and Ibeabuchi, 2010).

Antioxidants are important in the prevention of oxidative damage reported to be responsible for various diseases such as cancer, cardiovascular diseases, diabetes, cataracts, premature aging, wrinkling of the skin, arthritis, Alzheimer's disease and much more in human (Azuma *et al*., 1999; Agoreyo *et al*., 2003). Secondary metabolites such as flavonoids, which are one of the bioactive constituents present in plants, have been reported to have strong antioxidant activity. These naturally occurring antioxidants have also been reported to show antimicrobial, anticancer, anti-inflammatory activity ((Hertog, et al., 1993; Cushnie and Lamb, 2005). This study was therefore carried out to determine the bioactive compounds that are present in the two varieties of *Adenia lobata* that are used at different stages of treatment for diseases such as cancer, in some places in the eastern part of Nigeria and also to determine the antioxidant activity of their various solvent fractions.

MATERIALS AND METHODS

Plant Materials

The leaves of the two varieties of *Adenia lobata* were obtained from Abia state, Nigeria. These two varieties were *Adenia lobata* with cordate leaves (ALC) and Adenia lobata with palmate leaves (ALP). These plants were authenticated at the Deparment of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City and the Forest Research Institute of Nigeria (FRIN), Ibadan; where herbarium voucher specimens FHI NO. 108783 for ALC and FHI NO. 108459 for ALP were deposited.

Preparation of Crude Extract
The plant materials were air dried on the laboratory bench and further dried in the oven for 1 hr at a temperature of 37^{o}C. The dried leaves were converted to pulverized form using mortar and pestle. A quantity (850g) of each plant sample was soaked in 80% MeOH – H_2O [80% methanol – 20% water (v/v)] for 72 hr. The crude extract (methanolic extract) obtained after filtration was concentrated by rotary evaporator and then freeze dried. A portion (40g) of the concentrated methanolic extract from each plant was dissolved in distilled water and poured into a separatory funnel. The content of the separatory funnel was extracted with 400 mL each of hexane, dichloromethane (DCM), ethylacetate and butanol respectively; to obtain the various solvent fractions, which were concentrated using rotary evaporator and used for the determination of antioxidant activity. The antioxidant activity of the freeze dried aqueous extract was also determined.

Phytochemical Screening
The methanolic extract of each plant was analysed to determine the presence of bioactive compounds. Alkaloids, saponins, anthraquinones, flavonoids, tannins, steroidal glycosides, cardiac glycosides, carbohydrates were determined according to the methods of Harborne (1973); Odebiyi and Sofowora (1978) and Sofowora (1992).

Paper Chromatography
Paper chromatography was further used to confirm the presence of tannins in the plant samples. Catechol was used as the reference standard and the solvent system was
n-butanol, water and acetic acid in a ratio of 4: 1: 5.

Thin Layer Chromatography
Thin layer chromatography (TLC) was used to partially purify the crude extract that was obtained from each plant. Chromatography plates were coated with silica gel, dried and activated in the oven at 110^{o}C. The solvent system used was acetone, water, and 25% ammonia in a ratio of 90: 7: 3. The chromatogram was viewed under the UV lamp and sprayed with Dragendroff's reagent. A pink colour on the TLC plate under the UV lamp and after spraying with Dragendroff's reagent confirmed the presence of alkaloids.

Determination of Antioxidant Activity
Antioxidant activity of the various solvent fractions was determined by the diphenyl picryl hydrazyl (DPPH) method (Bandoniene *et al*., 2002).

RESULTS
Phytochemical screening of the methanolic extract of the two varieties of *Adenia lobata* showed the presence of alkaloids, carbohydrate, glycosides, saponin, flavonoids and tannins. In *Adenia lobata* with cordate leaves (ALC), cardiac and steroidal glycosides were present but *Adenia lobata* with palmate leaves (ALP) showed only the presence of steroidal glycosides. Brucine alkaloid was present in ALC, while it was absent in ALP (Table 1).

Table 1: Phytochemical constituents of two varieties of *Adenia lobata*

Bioactive compounds	*Adenia lobata* with cordate leaves (ALC)	*Adenia lobata* with palmate leaves (ALP)
Alkaloids	+	+
Brucine alkaloids	+	-
Carbohydrate	+	+
Reducing sugars	-	-
Cardiac glycosides	+	-
Steroidal glycosides	+	+
Anthraquinones	-	-
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Phlobatanins	+	+
Pyrogallol tannins	-	-

Keys: + = Presence of bioactive compound; - = Absence of bioactive compound

The R_f values of spots obtained from the paper chromatography that was carried out to confirm the presence of tannins in the two plant samples are shown in Table 2. Spraying of the paper chromatogram with ferric chloride also gave black spots that characterized the presence of tannins.

Table 2: R_f values of paper chromatographic spots of the two varieties of *Adenia lobata*

Sample	Rf value
Reference standard (Catechol)	0.94
Methanolic extract of ALC	0.87
Methanolic extract of ALP	0.90

ALC - *Adenia lobata* with cordate leaves, ALP - *Adenia lobata* with palmate leaves

Partial purification of each *Adenia lobata* variety gave two spots respectively and their various R_f values are shown in Table 3. These two spots were pink in colour under UV light and when sprayed with Dragendroff's reagent, which confirmed the presence of alkaloids in the two plant samples. The antioxidant activity of the various solvent fractions is shown in Table 4 and ethyl acetate fraction of ALC (*Adenia lobata* with cordate leaves) had the highest activity.

Table 3: **R_f values of TLC chromatographic spots of the two varieties of *Adenia lobata***

Sample	Rf value	
	Spot 1	**Spot 2**
Methanolic extract of ALC	0.73	0.83
Methanolic extract of ALP	0.82	0.93

ALC - *Adenia lobata* with cordate leaves, ALP - *Adenia lobata* with palmate leaves

Table 4: Antioxidant activity of the solvent fractions of the two varieties of *Adenia lobata*

Code	Solvent fraction	Antioxidant activity (%)
ALC	Crude extract (methanolic)of ALC	-
ALP	Crude extract (methanolic)of ALP	-
HALC	Hexane fraction of ALC	-
HALP	Hexane fraction of ALP	6.08
DALC	Dichloromethane fraction of ALC	-
DALP	Dichloromethane fraction of ALP	32.95
EALC	Ethylacetate fraction of ALC	63.87
EALP	Ethylacetate fraction of ALP	-
BALC	Butanol fraction of ALC	43.12
BALP	Butanol fraction of ALP	28.94
AALC	Aqueous fraction of ALC	-
AALP	Aqueous fraction of ALP	-

DISCUSSION

The various phytochemical constituents (Tables1-3) that were detected in the two varieties of *Adenia lobata* that were used in this study have beneficial medicinal importance (Newman and Cragg, 2007; Ekhaise *et al*., 2010). Alkaloids have been reported to have analgesic, anti-hypertensive and anti-microbial activities (Mallikharjuna *et al*., 2007; Zongo *et al*., 2009). Cardiac and steroid glycosides are used in the treatment of congestive heart failure and arrhythmia (Dewick, 2002). Saponins have anti-inflammatory, anti-melanogenic and anti-spasmodic activities that could protect against skin cancer and inhibit human colon cancer (Corea *et al*., 2005; Kawabata *et al*., 2011). Flavonoids have strong antioxidant effect and show anti-allergic, anti-inflammatory, anti – microbial and anti – cancer activities (Kim *et al*., 2004; Cushnie and Lamb, 2005; Aregullin *et al*., 2006). Tannins have also been reported to exhibit anti-viral, anti-bacterial and anti-tumor activities (Akiyama *et al*., 2001; Lu *et al*., 2004). The presences of these bioactive agents in these plants confer on them therapeutic activities that enable them to be used as traditional medicine in several countries in Africa, such as Nigeria.

Adenia lobata with cordate leaves (ALC) showed the presence of more bioactive agents than *Adenia lobata* with palmate leaves (ALP) (Table 1). The presence of more phytochemical constituents in ALC makes it a stronger traditional medicine and it is therefore used during the first phase of treatment for cancer, in eastern Nigeria; while ALP is used during the second phase of treatment.

Antioxidant activity was found in various solvent fractions of the two varieties of *Adenia lobata* that were used in this study (Table 4). Ethylacetate and butanol fractions of ALC possessed antioxidant activity; while antioxidant activity was found in hexane, dichloromethane and butanol fractions of ALP. The highest antioxidant activity occurred in the ethylacetate fraction of ALC. The antioxidant activity of these solvent fractions that were obtained from the two varieties of *Adenia lobata* is due mainly to the free radical – scavenging activity of their phenolic compounds such as flavonoids and tannins (Rahman and Moon, 2007; Hasan *et al*., 2009).

Oxidative damage by free radicals have been reported to be the fundamental mechanism underlying various diseases (Aruoma, 2003). For instance, in carcinogenesis, reactive oxygen species (ROS) are responsible for initiating this process by causing DNA damage (Tsao *et al*., 2004).

Since naturally occurring antioxidants such as flavonoids have been reported to have anticancer activity against human breast cancer cell lines by inhibiting their proliferation (Du *et al*., 2010; Rahman *et al*., 2011); then the presence of antioxidant activity in the various solvent fractions of *Adenia lobata* varieties, reveals why these plants are used for the treatment of cancer in some places in eastern Nigeria.

CONCLUSION

This comparative study on the two varieties of *Adenia lobata*, therefore showed that ALC, aside from having the highest antioxidant activity also have more phytochemical constituents than ALP; which reveals why it is employed in first - line treatment of cancer in some places in eastern Nigeria. Further studies are ongoing to determine the specific bioactive compounds that are present in these plants; which are responsible for their antioxidant activity and thus their anticancer activity.

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