

## PHYTOCHEMICAL SCREENING AND ANTI-CANCER STUDY OF ETHYLACETATE EXTRACTS OF TWO AFRICAN *Tapinanthus* SPECIES

A. A. Azeez<sup>1,2\*</sup>, A. Bhatnagar<sup>2</sup> S. H. Makana<sup>3</sup>, C. C. Mandal<sup>3</sup>, V. Kumar<sup>2</sup> and S. A. Aboaba<sup>1</sup>

1: Department of Chemistry, University of Ibadan, Ibadan, Nigeria

2: Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Ajmer

3. Department of Biochemistry, School of Life Sciences, Central University of Rajasthan, Ajmer

[habeebullah2010@gmail.com](mailto:habeebullah2010@gmail.com) +2348060317308

### ABSTRACT

Cancer- a multifaceted disease- is one of the leading causes of mortality in the world and breast cancer is currently a significant global health issue. Cancer cells in general have developed resistance to most of the existing therapies. *Tapinanthus* species have been reported for treatment cancer by traditional healers in Africa. For this study, ethylacetate extracts of *Tapianthus buntigii* growing on *Citrus paradise* and *Tapinanthus dodoneifolius* hosted by *Parkia biglobosa* were screened for phytochemical constituents and anticancer potentials against MDA-MB-231, an a aggressive cancer cell line using MTT assay. The result of phytochemical screening revealed presence of steroids, saponins and cardiac glycosides but absence of tannins, terpenoids, alkaloids, and flavonoids. The extracts showed comparable anticancer activities against the cell line. From the results of this study, it is concluded that the *Tapinanthus species* could be reservoirs for potent anticancer drugs or lead compounds.

**Keywords:** Cancer, *Tapinanthus* species, MDA-MB-231, Phytochemicals, MTT assay

### INTRODUCTION

Cancer is a disease associated with uncontrollable growth of the cells. The cells spread quickly and widely invading the other parts of the body [1]. Cancer disease is one of the most significant public health issues in all economic standards [2]. It is life threatening and second reason of death after heart disease universally [3]. There are several forms of cancer and they are named in relation to the parts of the body they affect. Breast cancer is regarded as the major cause of cancer-related mortality and frequent malignancies among women globally [4]. It is the most commonly

diagnosed (6.9%) after lung (11.4%) and colorectal (10.0%) [5].

Resistance of cancer to medication is growing at an alarming rate and calls for development of more effective anticancer agents [3]. Natural products and their derivatives have been identified historically as major contributors to pharmacotherapy, especially in the treatment of cancer and infectious diseases [6].

Plants play significant roles in cancer therapy. They are reservoirs of natural molecules which can induce apoptosis in cancer cells by exerting

influence on molecular mechanisms implicated in cancer progression [7]. Many powerful anticancer drugs such as vinblastine, paclitaxel were firstly isolated from plant materials [8].

*Tapinanthus* species -hemi-parasitic plants that grow on diverse hosts in African continents- are locally referred to as all-purpose herbs because they are traditionally used to treat various diseases such as diabetes, cancer, inflammation, hypertension, malaria, anemia, anxiety, itching, and several other ailments [9]. *Tapinanthus dodoneifolius* leaves and twigs are used in the treatment of various ailments in different areas around the world. It is used in the Northern Nigeria in the treatment of stomach ache, diarrhea, dysentery, wound and cancer [10]. Aqueous extracts of the leaf of *T. butungii* induced excellent dose-dependent reduction in blood glucose concentration of hyperglycaemic, alloxan induced diabetic and normoglycemic rats. This may suggest that *T. butungii* could, at least partially stimulate insulin production and glucose utilization, like chlorpropamide and glibenclamide, to cause its hypoglycaemic effect [11].

## MATERIALS AND METHODS

### *Sample Collection and Authentication*

Leaves of *Tapinanthus dodoneifolius* growing on *Parkia biglobosa* and *Tapinanthus butungii* growing on *Citrus paradise* were collected from Otefan village, Oyo, Oyo State, Nigeria and

Citrus orchard of National Horticultural Research Institute, Ibadan, Oyo State, Nigeria, respectively. The *T. dodoneifolius* and *T. butungii* were identified and authenticated at the herbaria of the University of Ibadan, Nigeria and Forest Research Institute of Nigeria, Ibadan Nigeria, respectively. The two plants were assigned with voucher numbers UIH-22938 and FHI-112612 respectively.

### *Sample Preparation*

Leaves of the plants were plucked, washed, air-dried, pulverized and kept in air-tight polythene bags prior to period of extraction.

### *Extraction*

The pulverized leaves were firstly defatted by petroleum ether and then followed by cold maceration with ethyl acetate at room temperature for 72 hours to obtain ethylacetate extracts.

### *Phytochemical Screening*

Qualitative phytochemical screening was carried out the ethylacetate leaf extracts of *T. dodoneifolius* and *T. butungii* were screened qualitatively for tannins, terpenoids, alkaloids, steroids, saponins, flavonoids and cardiac glycosides using standard methods in the literature [12, 13, 14, 15 and 16].

### *Anticancer Study*

To assess anti-cancer activity of the plant extracts, MTT assay was performed. The impact of these extracts on MDA-MB-231 cell viability was done by seeding  $10 \times 10^4$  MDA-MB-231 cells in a 96-well plate in 10% DMEM. The treatment of various plant extract was administered to the cells and incubated for 24 hours in a 5% CO<sub>2</sub> incubator, after which 10 µL MTT solution (5

mg/mL) was added and the cells were incubated at 37°C for 2 hours. To dissolve the formazan crystals generated during MTT reduction, DMSO is added which leads to purple colouration being developed. Microplate reader is used to observe the absorbance of the sample at 530nm and calculations were done [17].

## RESULTS AND DISCUSSION

| Phytochemicals     | Ethylacetate |     |
|--------------------|--------------|-----|
|                    | TBF          | TDI |
| Tannin             | -            | -   |
| Terpenoid          | -            | -   |
| Alkaloid           | -            | -   |
| Steroid            | +            | +   |
| Saponin            | +            | +   |
| Flavonoid          | -            | -   |
| Cardiac glycosides | +            | +   |

**TBF:** *Tapinanthus butingii* growing on *Citrus paradise* **TDI:** *Tapinanthus dodonefoliuos* growing on *Parkia biglobosa*

+: Present, -: Absent

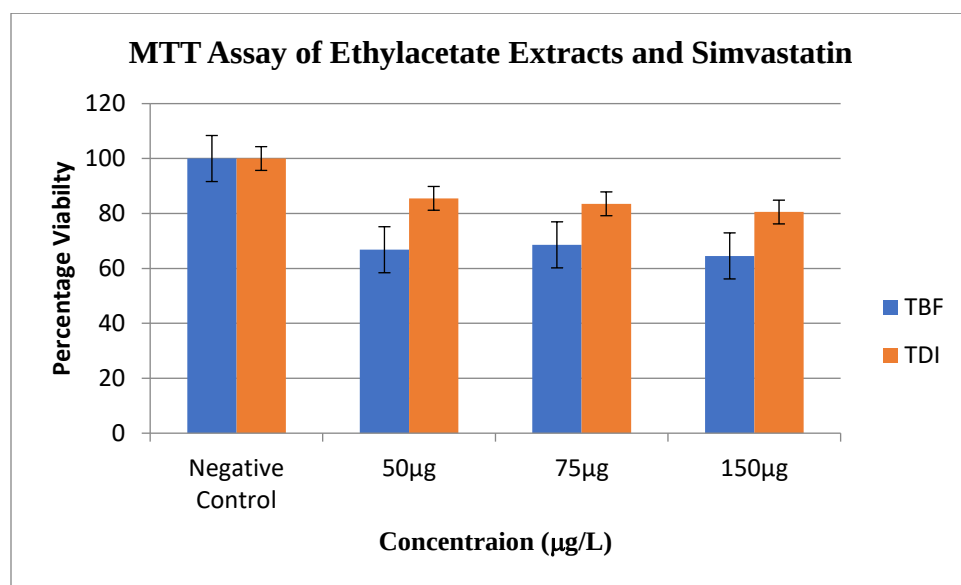
Table 1 revealed that ethylacetate extracts of the two plants exhibited the same phytochemical profiles; steroid, saponin and cardiac glycosides were present while tannin, terpenoids, alkaloid, and flavonoids were found to be absent. The present phytochemicals; steroid, saponin and

cardiac glycosides have been reported to possess many medicinal properties. Steroids are naturally occurring organic compounds with a great variety of different biological functions [18]. Cortisol, an important steroid is found useful in the treatment of skin ailments, asthma, and inflammation from

arthristhis [19]. 1,25(OH)<sub>2</sub> D is the biologically active form of vitamin D- a secosteroid- and its analogs have been reported to prevent cancer development or retard its metastasis or progress [20]. Saponins-a group of compounds characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains- are on consumer demand for their physicochemical (surfactant) properties and mounting evidence on their biological activity such as anticancer and anticholesterol activity [21].

They have demonstrated great therapeutic potentials such as cytotoxic, hypolipidemic, antioxidant, hypoglycaemic, anti-hypertensive,

anti-asthmatic and anti-microbial activities [22 and 23]. Some saponins such as methyl protoneograccillin, protodioscin, methyl protograccillin and protoneodioscin have been recommended by National Cancer Institute as potent anti-carcinogenic agents [24]. Cardiac glycosides-a unique group of secondary metabolites- have been considered as one of the most useful drugs in therapeutics [25]. Several cardiac glycoside metabolites have been previously reported to exhibit promising pharmacological activities such as anti-inflammatory, anti-viral, cytotoxicity, enzyme-inhibitory activity, neurotoxic, and markers against coronary heart diseases [26].



**TBF:** *Tapinanthus butingii* growing on *Citrus paradise* **TDI:** *Tapinanthus dodonefoliuos* growing on *Parkia biglobosa*

Figure 1 shows that the two extracts show anticancer properties against MDA-MB-231 cell line. Ethylacetate extract of *Tapinanthus*

*butingii* (with an IC<sub>50</sub> of 14.7623 µg/L) growing on *Citrus paradise* demonstrated a non-dose

dependent activity while ethylacetate extract of *Tapianthus dodoneifolius* (with an  $IC_{50}$  of 76.7695  $\mu\text{g/L}$ ) hosted by *Parkia biglobosa* showed a dose dependent concentration. Presence biologically active phytochemicals such as saponins, steroids and cardiac glycosides in the extracts may be responsible for their promising anticancer potentials. The phytochemical may be showing the activities in synergistic manner or individually.

## CONCLUSION

Qualitative analysis of the two *Tapianthus* species revealed the presence steroid, saponin and cardiac glycoside and the two species demonstrated good anticancer activities against though *T. butingii* exhibited better activities than *T. dodneifolios*. The two *tapianthus* species could be reservoirs for potential anticancer drugs or lead compounds.

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