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Phytochemical Characterization of Dichloromethane Stem Bark Extract of *Piliostigma thonningii* (Schum.)

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Abstract:- *Piliostigma thonningii* (Schum.) commonly referred to as monkey bread belongs to Fabaceae family and is used in the tropics for medicinal and nutritional uses. The plant is utilized for management of human ailments among them rheumatism, malaria, fever, snake bites and dysentery. It is widely acknowledged that plant pharmacological activities are driven by their secondary metabolites. Identification of the metabolites has led to drug discovery and leads for designing of novel compounds to enhance human health. Though *P. thonningii* is endowed with numerous bioactivities, there is minimal literature detailing the phytochemicals responsible for its therapeutic efficacies. For that reason, the present embarked on quantitative phytochemical characterization of DCM stem bark extract of *P. thonningii.* The analysis was carried out using gas chromatography-mass spectrometry. The results showed that the extract contained various classes of phytocompounds including flavonoids, phenolics, phytosterols and fatty acids. The most abundant compounds found in the extract were oleic acid and squalene whereas valencene and α-cedrene were the least concentrated. The compound identified in the extract have been reported to possess numerous pharmacological efficacies such as analgesic, anti-inflammatory, anticancer and antidiabetic activities. Thus, the promising therapeutic potential of *P. thonningii* is attributed to these phytocompounds. The study recommends further research to isolate and purify the activity ingredient from *P. thonningii* as well as determine their mechanism of action.

Keywords:- *P. thonningii, Phytochemicals, gas chromatography-mass spectrometry.*

I. INTRODUCTION

Botanicals with therapeutic properties have been utilized in health care since the birth of human civilization (Moni *et al.,* 2021). Globally, numerous efforts have been enlisted to investigate the effectiveness of plant-based medicines with some leading to discovery of drugs of widespread significance (Goni *et al.,* 2021). Herbal medicine offers an alternative avenue for healthcare as the global burden of disease continues to grow. Search of new therapeutics from plant source is an active area of research as new treatment intervention is required to grapple with existing and emerging threats to human health (Reza *et al.*, 2021). Past evidences have indicated that plant species have numerous pharmacological properties and a large number of them are already being applied for treatment of various human ailments including cancer, diabetes, pain, arthritis, malaria (Martínez et al., 2020; Reza *et al.*, 2021).

The enormous health benefits associated with herbal produced is accredited to the phytochemicals they contain (Reza *et al.*, 2021). The phytochemicals have a wide array of applications including nutraceuticals, drug manufacturing industries, food supplements, medicine and chemical leads for synthetic drug design (Pollio *et al.*, 2016; Ibrahim *et al.*, 2023). The main phytochemicals present in plants include alkaloids, flavonoids, phenolic acids, terpenoid, sterols and triterpenes (Reza *et al.*, 2021). Alkaloids are known have analgesic and antimicrobial properties (Saranraj and Sivasakthi, 2014), glycosides can moderate blood pressure (Han *et al.*, 2007), phenolic compounds are known to possess various biological activities including apoptosis, anti-aging, anti-inflammation, anti-oxidant, anti-atherosclerosis, inhibition of angiogenesis, (Shrestha *et al.*, 2015) and others, saponins have anti-inflammatory and antibacterial potency (Chang *et al.*, 2023), terpenoids exhibits antimicrobial and antimalaria activities (Mahizan *et al.*, 2019).

Though there is enormous literature regrading phytochemical composition of medicinal plants, little is known regarding the phytochemistry of *Piliostigma thonningii*. Consequently, this study aimed to investigate the chemical constituents of dichloroethane (DCM) stem bark extract of *P. thonningii* as a potential source of lead compounds for developing safe, efficacious, and affordable therapeutics. *Piliostigma thonningii* (Fig 1), an evergreen shrub, belongs to Fabaceae family, sub family Caesalpinioideae. It natively grows in the tropics of Africa. Its common names include monkey bread, camel's foot, wild

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bauhinia, (English) and *mchikichi* (Swahili) (Orwa *et al*., 2009). The plant bears hard and hairy fruits. Its fruit pulp is rich in organic acids, proteins, fibre, vitamins, calcium oxalates, starch and essential oils (Orwa *et al.*, 2009). Its traditionally used to treat and manage ulcers, pain, wounds, arthritis, fever, malaria, sore throat, diarrhea, gingivitis, respiratory conditions (Ighodaro and Omole, 2012; Afolayan *et al.*, 2018). Its crude extracts have been demonstrated to exert antibacterial (Akinpelu and Obuotor, 2000), anti-lipidemic (Ighodaro and Omole 2012), anti-inflammatory (Ibewuike *et al.*, 1997) and anti-helminthic (Asuzu and Onu, 1994) activities.

Fig 1 *Piliostigma thoningii* (Schumach)

II. MATERIALS AND METHODS

A. Collection of Plant Material

Stem barks of *P. thonningii* were collected from Mbeere North sub-County, Embu County in Kenya. The plant materials were authenticated by a taxonomist at the Department of Plant Sciences, Kenyatta University (Voucher Specimen Number GM001/2017). The samples were cleaned with tap water, rinsed with distilled water, then chopped into small pieces. After this, the pieces were shade dried on a laboratory bench. After completely drying, they were milled into powder with an electric mill. The resulting stem bark powder was packaged into labeled khaki sack and stored at 25 ± 2 ^{o}C.

B. Preparation of Plant Extracts

The powdered plant material (200 g) was soaked in 750 ml of analytical-grade dichloromethane (DCM) in a conical flask and covered with aluminum foil. The mixture was left to stand for 48 hours during which it was shaken periodically and stirred with a glass rod. Thereafter, the extract was filtered through Whatman filter papers (No.1) and concentrated *in vacuo* using a rotary evaporator at 28 ^{0}C. The resulting crude extract was transferred into clean, dry, pre-weighed glass bottles and completely air-dried at room temperature.

C. Phytochemical analysis of the DCM stem bark extract of P. thonningii

The bioactive constituents of DCM stem bark extract of *P. thonningii* were characterized using Gas Chromatography - Mass Spectrometry (GC-MS) following published protocols (Hossain *et al.*, 2020; Ibrahim *et al.*, 2021).

III. RESULTS AND DISCUSSION

➢ *Phytochemistry of P. thonningii*

The results revealed various phytocompounds of various classes including fatty acids, triterpenoids, phytosterols, phenols, whereby oleic acid (218.60±15.6 ng/g) and 1-nonadecene (33.34±2.38 ng/g), and squalene (31.45±2.25 ng/g) were the most abundant and carvacrol (1.37±0.10 ng/g), valencene (0.72±0.05 ng/g), and α-cedrene (0.69±0.05 ng/g) were the least abundant (Table 1).

Table 1: Phytochemical composition of the DCM stem bark extract of *P. thonningii*

Compound name	Molecular formula	Chemical class	Concentration (ng/g)
Oleic Acid	$C_{18}H_{34}O_2$	Fatty acid	218.60±15.6
Squalene	$C_{30}H_{50}$	Triterpene	31.45±2.25
Alpha Amyrin	$C_{30}H_{50}O$	Triterpenoid	28.88±2.06
Decosanoic acid	$C_{22}H_{44}O_2$	Fatty acid	23.61±1.69
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acid	21.88±1.56
Gamma Tocopherol	$C_{28}H_{48}O_2$	Phenolic	9.52±0.68
Gamma Sitosterol	$C_{29}H_{50}O$	Phytosterol	5.85±0.42
Campesterol	$C_{28}H_{48}O$	Phytosterol	5.50±0.39
Palmitoleic acid	$C_{16}H_{30}O_2$	Fatty acid	4.28±0.306
4-(2,4,4-trimethylpentan-2-yl) phenol	$C_{14}H_{22}O$	Phenolic	2.38±0.17
Pentadecanoic acid	$C_{15}H_{30}O_2$	Fatty acid	2.27±0.162
Stigmasterol	$C_{29}H_{48}O$	Phytosterol	1.49±0.12
Carvacrol	$C_{10}H_{14}O$	Phenolic	1.37±0.10
Valencene	$C_{15}H_{24}$	Sesquiterpene	0.72±0.05
α-cedrene	$C_{15}H_{24}$	Sesquiterpene	0.69±0.05

Other studies have reported similar findings on the classes of secondary metabolites present in *P. thonningii.* Moriasi *et al.* (2020) reported that *P. thonningii* methanolic and aqueous stem bark extracts contains glycosides, phenols, steroids, saponins, and flavonoids. Further, flavonoids, saponins and terpenes were confirmed resent in ethanol extract of *P. thonningii* extract (Egharevba and Kunle, 2010). Phytochemical analysis of *P. thonningii* roots showed it contained alkaloids, flavonoids, terpenoids, tannins, phenols, saponins and steroids (Alagbe, 2019).

Bioactivities of some of the compounds identified in *P. thonningii* have been reported in literature. Fatty acids such as oleic and palmitoleic acid has been reported to possess antioxidant, anti-inflammatory and anti-Alzheimer activities (Mazouz *et al.,* 2020; Agidew *et al.,* 2021; Santa-María *et al.,* 2023). Squalene, a triterpene, has been show to exhibit cardioprotective, anticancerous and antioxidant properties (Kumar *et al.,* 2023). Triterpenoid alpha amyrin, has been indicated to exert anti-microbial, antitumor, anti-inflammatory, anxiolytic, and hepatoprotective potencies (Viet *et al.*, 2021).

Gamma Tocopherol, a phenolic compound, has been reported to possess antioxidant, anti-inflammatory, antidiabetic, anticancer, anti-nociceptive activities (Ebrahimi *et al.,* 2021; Zakaria *et al.,* 2021). Another phenolic compound, carvacrol has been revealed to possess a wide array of bioactivities among them; anti-inflammatory, immunomodulation, antioxidant, antifungal and antibacterial efficacies (García-Risco *et al.,* 2011).

Valencene and α-cedrene, sesquiterpenes, have been noted to possess a wide range of pharmacological activities. Multiple lines of research have reported that Valencene has antimicrobial, antiparasitic, antitumor, antinociceptive, anti-inflammatory and antioxidant activities (Yamaguchi, 2019; Zhang *et al.,* 2022). α-cedrene has been indicate to mediate anticancer and antimicrobial activities (Su *et al.,* 2012). The compound has been demonstrated to offer remedial efficacies against hepatic steatosis (Tong *et al.,* 2017). Moreso, α-cedrene has been shown to reverse obesity pathophysiology in rats (Tong *et al.*, 2019).

Many phytosterol: gamma sitosterol, campesterol and stigmasterol were present in the studied extract and have associated with a wide range of pharmacological activities. These compounds have been revealed anti-cholinesterase, antioxidant and anti-inflammatory activities (Elufioye, 2017; Ayaz *et al.,* 2017; Karimi *et al.,* 2021). The compounds are also known to reduce plasma cholesterol levels thereby reducing the risk of obesity cardiovascular complications (Miras-Moreno *et al.,* 2016). Besides, phytosterols possess anticancer and antidiabetic efficacies (Miras-Moreno *et al.,* 2016).

IV. CONCLUSION

The findings of the present study demonstrated that *P. thonningii* DCM extract contains constituents with promising therapeutic potential. Multiple previous studies confirmed the therapeutic activities of the identified compounds. Thus, the therapeutic potential associated with *P. thonningii* is mediated by its phytochemical constituents. Based on the study findings *P. thonningii* may provide dependable reservoir for therapeutic agents.

DECLARATIONS

➢ *Author Contribution Statement*

All the authors conceived the research idea. Farida Moraa Okioga performed the experiments, analysed the data, and drafted the manuscript under the supervision of Dr Mathew Piero Ngugi and Dr Huxley Mae Makonde. All the authors reviewed and approved the final version of the manuscript for submission.

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➢ *Competing Interest Statement*

The authors declare no conflict of interest.

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