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Investigation of natural product constituents from Pergulariatomentosa. A means of exposing final year chemistry students to basic practical knowledge

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ABSTRACT
Natural products have been a major source discovery for existing and emerging diseases. Pergulariatomentosa is used as remedy for different kinds of health conditions. This study is designed to evaluate the phytochemical constituents present in the leaf of P.tomentosa. The phytochemical screening of extracts of P. tomentosa was performed using standard methods. The presence of terpenoids, alkaloids, tannins, glycosides, cardiac glycoside, saponin and anthraquinone glycosides were detected in the plant extracts. The isolation, characterization and structural elucidation of individual phytochemicals as well as in silico, in vitro and in vivo studies are recommended for further studies.

Keywords: Natural products;Pergulariatomentosa; phytochemical screening

I. INTRODUCTION

Natural products have gained widespread relevance as an effective and alternative source of healthcare with excellent biological and pharmaceutical properties (Atanasovet al., 2015). The emerging world population uses their knowledge, beliefs, theories and indigenous experiences to prescribe medicinal plants that can prevent, treat and improve their mental and physical illnesses (Tesfahuneygn and Gebreegziabher, 2019). Globally, more than 80,000 flowering plants are used for various medicinal purposes worldwide (Jamshidi-Kiaet al., 2018). In Nigeria, about 80% of people living in the rural and sub-urban communities depend on medicinal plants for their primary health care (Adenugaet al., 2020)

Phytochemicals are secondary metabolites obtained from plants. Studies have showed that the medicinal relevance of plants is attributed to the presence and abundance of secondary metabolites (Kennedy and Wightman, 2011). For instance, the presence of polyphenolic chemical constituents in medicinal plants helps to scavenge free radical produced from oxidative stress (Soni and Sosa, 2013). Hence, phytochemicals are useful for treating diseases oxidative related diseases. Also, terpenoids, alkaloids, tannins, cardiac glycosides and saponins are proven therapeutic arsenals against diabetes, malarial, cancer, neural disorders and other life-threatening diseases (Abdi, 2017)

Pergulariatomentosa, also known as milk weed, is a medicinal plant that belongs to the Apocynaceaefamily (Chandiet al., 2018). The plant is a perennial herb with about 30 cm at maturity. It is widely found the Middle East, Gulf region and the Asian countries. Also, P. tomentosa is endemic in north Sudan, Ethiopia, Kenya, Algeria, Egypt and the north central part of Nigeria (Ketaet al., 2016; Ntie-Kang and Yong, 2014). Ethnomedicinally, P. tomentosa is used in the treatment of asthma, rheumatic fever, helminthiases, skin infection, bronchitis headache, constipation and tuberculosis (Al-Mekhlafi and Masoud, 2017). Pharmacologically, the antioxidant, antibacterial, cytotoxic, antifungal and antipoliferative activities have been evaluated (Hassan et al., 2007; Lahmaret al., 2017). Despite the vast investigation of phytochemical and pharmacological properties of different morphological properties of P. tomentosa, the phytochemical composition of the plant native to

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the inhabitants of the north central part of Nigeria is yet to be evaluated.

This study aimed to evaluate the phytochemical composition of P. tomentosa in order to understand the distribution of secondary metabolites in the plant and expose final year chemistry students to basic practical knowledge in natural product chemistry.

II. MATERIALS AND METHODS

2.1 Plant collection and preparation

The leaf of P. tomentosa was collected at Nagazi area,Okene metropolis in Kogi State, Nigeria. The plant material was identified, authenticated and voucher specimen was deposited at Federal college of Education, Okene herbarium. Thereafter, the leaves were air-dried to preserve the chemical compositions. The dried leaves was pulverized and stored in airtight bag for future purposes.

2.2 Extraction

1000 g of pulverized P. tomentosa leaf was extracted successively in n-hexane, ethanol and water in order of increasing polarity. The n-hexane, ethanol and aqueous solution was filtered and concentrated in vacuo to afford the n-hexane (8 g), ethanol (18.5 g) and aqueous (40 g) extracts.

2.3 Phytochemical screening

The phytochemical screening of the extracts of P. tomentosa was carried out by adopting the standard procedure as described by Trease and Evans. 2009. The procedures of the qualitative tests conducted are discussed below:

Test for saponins

One mL of the P. tomentosa extractswere diluted with distilled water to 20 mL and shaken in a graduated cylinder for 15 minutes. The formation of one centimeter layer of foam indicates the presence of saponins.

Test for cardiac glycoside

Two millilitres (2 mL) of the n-hexane, ethanolic and aqueous extracts of P. tomentosawas added into 3 drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5 mL of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride; this was transferred to the surface of 2 mL concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown colouration respectively as indicative of the presence of cardiac glycosides.

Test for glycosides

A small amount of n-hexane, ethanolic and aqueous extracts of P. tomentosawas taken in 1 mL of water in a test tube and a few drops of aqueous sodium hydroxide (NaOH) were added. A yellow coloration indicates the presence glycosides.

Test for anthraquinone glycoside

To the 3 mL of n-hexane, ethanolic and aqueous extracts of P. tomentosa, dilute $H_2SO_4$was added. The solution was then boiled and filtered. The filterate was cooled and equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of ammonia solution was added to the organic layer. The ammonia solution turned pink showing the presence of anthraquinone glycoside.

Test for flavonoids

One to five drops of concentrated hydrochloric acid (HCl) were added to little amount of n-hexane, ethanolic and aqueous extracts of P. tomentosa. An immediate development of a red colourshows the presence of flavonoids.

Test for alkaloids

Two mL of n-hexane, ethanolic and aqueous extracts of P. tomentosawas taken in a test tube and then 0.2 mL HCl was included, followed by 1 mL of Meyer's reagent. A yellowish coloration indicates alkaloid's presence.

Test for tannins

Five mL of the n-hexane, ethanolic and aqueous extracts of P. tomentosawas placed in a test tube and then 2 mL of 5% of $FeCl_3$ solution was added. A greenish-black precipitate indicates the presence of tannins.

Test for terpenoids

In a test tube containing 2 mL of chloroform, 0.5 mL of n-hexane, ethanolic and aqueous extracts of P. tomentosawas added. This is then followed by the addition of 3 mL conc. H_2SO_4 which forms a layer. The formation of reddish brown coloration at the interface shows the presence ofterpenoids.

III. RESULTS AND DISCUSSION

The presence of phytochemicals has been identified as the major factor responsible for the therapeutic properties of medicinal plants. Individual phytochemical isolated from medicinal plants has proven their effectiveness in treating oxidative stress related and various life threatening diseases that continued to pose serious health concern to the world inhabitants. In this study, phytochemical screening was carried out to elucidate the classes of phytochemicals present in the n-hexane, ethanolic and aqueous extracts of P.

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tomentosa endemic to north central region of Nigeria. The results obtained are shown in table 1.

Table 1: Phytochemical screening of different solvent extracts of P. tomentosa

S/N	Class of phytochemical	n-hexane	Ethanol	Aqueous
1	Terpenoid	++	++	_
2	Alkaloid	+	++	+
3	Glycoside	-	+	++
4	Cardiac glycoside	-	+	++
5	Saponin	++	+	+
6	Flavonoid	-	++	++
7	Tannin	-	++	++
8	Anthraquinone glycoside	-	+	++

Plus (+) signifies detected or presence; Plus (++) signifies strongly detected or present and minus (-) signifies absence.

The qualitative phytochemical screening revealed the abundance of saponin and terpenoids in the n-hexane extract of P. tomentosa. Also, the presence of alkaloid was slightly detected in the study. Furthermore, tannins, terpenoids, alkaloids and flavonoid were strongly observed in the ethanolic extract while cardiac glycoside, glycoside, saponin and anthraquinone glycoside were also observed. The aqueous extract has the abundance of flavonoids, tannins, anthraquinone glycosides and glycosides, while saponins and alkaloids were also detected.

The abundance of terpenoids and saponin in the n-hexane extract of P. tomentosa is attributed to their non-polar nature and that of the extracting solvent. Conversely, the ethanolic extract has the potential of extracting both polar and non-polar constituents. Hence, all the classes of phytochemical constituents tested were observed in the ethanolic extract. Furthermore, there were no traces of terpenoids in the aqueous extract because of its non-polar nature relative to the polarity of water as an extracting solvent.

The presence of flavonoids, tannins, anthraquinone glycosides, glycosides, saponins and alkaloids in the aqueous extract is in strong agreement with results obtained for P. tomentosa leaves harvested in Katsina, north western Nigeria (Ketaet al., 2016). Flavonoids, glycosides and tannins are secondary metabolites that possess free-radical scavenging, antidiabetic, antimicrobial, anticancer, antipoliferative and antimalarial activities (Nyamaiet al., 2016). The presence of these classes of compounds in P. tomentosa indicated that the ethanolic and aqueous extract may be useful in the treatment of diseases caused by accumulation of free radicals. Also, saponins and terpenoids has been identified as promising antimicrobial, anti-inflammatory, antidiabetic, antimalarial and cytotoxic activities, thereby indicating that the n-hexane extract may possess these interesting biological activities (Wang et al., 2012).

IV. CONCLUSION

The study has established the presence of secondary metabolites such as terpenoids, saponins, cardiac glycoside, glycosides, anthraquinone glycosides, tannins and flavonoids in the n-hexane, ethanolic and aqueous extracts of P. tomentosa. Phytochemicals that belong to these classes act as the therapeutic arsenals when the plant is taken as extract to treat different kinds of diseases. Hence, further studies are required to carry out chromatographic separation on the extracts in order to isolate individual chemical constituent based on their classes. The isolated chemical constituents can be characterized and elucidated so that their pharmacological properties can be fully studied through in silico, in vitro and in vivo methods.

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Conflict of interest

The authors declare no conflict of interest.

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