QUALITATIVE AND QUANTITATIVE ESTIMATION OF BIOACTIVE COMPOUNDS IN LEAVES OF TOUCH AND DYE PLANT (*MIMISA HAMATA*)

Abstract

The aim of this study is to determine the qualitative and quantitative bioactive compounds present in the leaf of touch and dye plant (Mimisa hamata). The leaf collected from Oko were washed, room dried and ground to powder. The ground sample was soaked in ethanol for 24 hours. After the contact elapsed the solvent were filtered and heated to obtain a crude extract. Qualitative analysis was carried out on the extract; while the quantitative analysis was carried out on the raw sample. The result showed that from the amount of precipitate formed and degree of colour change, it was deduced that the ethanolic extract contained all the seven phytochemicals analysed in the study (saponin, flavonoid, alkaloid, steroids, glycosides, phenol and tannin). The quaktitative analysis showed that touch and dye plant (Mimisa pigra) leaf contain flavonoid (243 ± 41 mg/dl), Alkaloids (1021 ± 13.2 mg/dl), steroid (1.01 ± 0.0 mg/100g), Phenol (674.23 ± 00 mg/100g), saponin (1.6 ± 0.1 mg/100g), Tannin (14.34 ± 0.4 mg/100g) and glycoside (0.01 ± 0.4 mg/100g). Phytochemical screening suggested that the presence of various phytochemicals in the leaf is responsible of its medicinal properties. The study recommend increased in the rate of touch and dye plant (Mimisa hamata) consumption among households and also industrial utilization of the leaf in formulation of new edible products is advised.

INTRODUCTION

1.1 Background of the study

Mimosa hamata (giant sensitive plant) is a creeping annual or perennial herb. It has been identified to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. *M. pudica* is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections. Phytochemical studies on *Mimosa pigra* have revealed the presence of alkaloids, non-protein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids (Geng *et al.,* 2007). Two well-known movements are observed in *Mimosa pigra* L. (uke in igbo): one is the very rapid movement of the leaves when it is stimulated by touch, heating, etc., and the other is the very slow, periodical movement of the leaves called nyctinastic movement which is controlled by a biological clock (Nascimento *et al.,* 2000).

The leaves of the sensitive plant M. pudica can adapt their closing response to electrical and mechanical stimulation so that they reopen to repeated stimulation. The more intense the stimuli and the longer the intertribal interval, the longer it takes to adapt. The *Mimosa pigra* plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since ancient times (Sun, *et al.*, 2002). Plant extracts or their active constituents are used as folk medicine in traditional therapies of about 80% of the world's population and Over 50% of all modern clinical drugs are of natural product origin. According to World Health Organization, medicinal plants is the best source to obtain a variety of drugs. The effect of plant extracts on microorganism have been studied by a very large number of researchers in different parts of the world and the use of a variety of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments (WHO, 2012).

Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body. The most important of these phytochemicals of plants are alkaloids, saponins, tannins, glycosides, flavonoids and phenolic components3. The

detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological effects (Nweze, 2010). The study of natural products on the other hand is called phytochemistry which are concerned with the enormous variety of organic substances (Primary and Secondary metabolites), that are elaborated and accumulated by plants and also deals with the chemical structures of these substances, their biosynthesis, turn-over, metabolism, their natural distribution and their biological function (Harbone, 2013). It is necessary to note that the products of primary metabolism e.g. Protein, sugar, fats etc are usually harmless except for some rare toxic protein and therefore of little interest to those investigating drug activity in plants (Sofowora, 2013).

There have been diverse speculations concerning the role of many of those secondary metabolites in the life of the plant but with a few exceptions. It is a major question in plant biochemistry which remains to be answered (Swain *et al*, 2009). Many different techniques may be followed in the screening of plants for products of pharmacological and chemical interest. Basic phytochemical screening consists of performing simple chemical test to detect the presence of alkaloids, tannins, saponins, flavonoids, digitalis glycosides etc in a plant extract (Sofowora, 2013).

1.3 Aim and objectives.

The aims of the study are to determine qualitative and quantitative bioactive compounds present in the leaf of touch and dye plant (Mimisa hamata).

The specific objectives of the study are:

1. To determine the qualitative phytochemical compositions of ethanol extract (Mimisa hamata).
2. To determine the quantitative phytochemical of (Mimisa hamata). leaves
3. Compare the phytochemical compositions of (Mimisa hamata). (giant sensitive plant) leaves with reported literatures

MATERIALS AND METHOD

Plant Materials

The plant materials used for this study was leaf of touch and dye plant were harvested from Oko, in Anambra State Nigeria. The leaves was dried under room temperature, ground into powder and then extracted with 80% ethanol.

Instruments/Equipment

The experiments was carried out with the following instruments: Adjustable micropipette (PERFECT USA), Refrigirator (Kelvinator, Germany), Centrifuge (Pic, England), automatic chemical balance (Gallenkamp, England), Water bath (Gallenkamp, England) and Spectrophotometer (UNICO 2100UV, England).

Chemicals and Reagents

All the chemicals used in this work was of analytical grade obtained from Merck, Germany; BDH chemicals Ltd, England; May and Baker Ltd, England.

Methods

Collection and identification Sample

The leaf of touch and dye plant was collected from Oko in Anambra State, and authenticated by a taxonomist in the, Department of Botany, Nnamdi Azikiwe University Awka.

Extraction of active principles of touch and dye plant

The leaf of touch and dye plant (Mimisa pigra) was harvested and dried at room temperature (29 – 35 ^{0}C) for three weeks, they was pulverized into powder with a Creston high speed milling machine. The powdered leaves (1 kg) was then macerated in 5 volume (w/v) 80% methanol at room temperature for 24 hours, The extracts was filtered through muslin cloth on a plug of wool in a glass column. The resulting methanol extracts was finely filtered with Whatman filter paper No 1, concentrated and evaporated to dryness using rotary evaporator at an optimum temperature of between 40 to 45 ^{0}C to prevent denaturation of active ingredients. The concentrated extracts was weighed, stored in clean bottles in the refrigerator in quanta at 0 ^{0}C or below until subsequently used.

Phytochemical Analysis

The qualitative and quantitative phytochemical analyses was carried out according to the method described by Harborn (1973) and Trease and Evans (1989).

Qualitative Phytochemical analysis

Test for Alkaloids

One gram of the extracts (1 g) was mixed with 1 ml of 1% HCl, warmed and filtered. Two milliltres (2 ml) of the filtrate was treated separately with Mayer's reagent. Turbidity or precipitation, green colour was observed to indicate the presence of alkaloids. Dragendorff's reagent, a red precipitate indicates the presence of alkaloid.

Test for Glycosides

Two milliters (2 ml) of the aqueous extracts was mixed with three milliters (3 ml) of chloroform and 1 ml of 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

Test for Saponins

The extracts was dissolved in 2 ml of boiling water in a test tube and allowed to cool. The cooled mixtures was thoroughly shaken to mix: The appearance of foam indicated the presence of saponin.

Test for Tannins (Ferric chloride method)

One gram (1 g) of the powdered extracts was boiled with 50 ml of water and filtered. 3 ml of the filtrates was added few drops of ferric chloride. Appearance of brownish green coloration showed the presence of tannin.

Test for Flavonoids

Two grams (2 g) of the extracts was dissolved in 5 ml of (95^0) ethanol and filtered. To (3 ml) of the rhizome filtrates was mixed with 4 ml of 1N NaOH in a test tube. Formation of dark yellow colour of the solution was observed which indicated the presence of flavonoids.

Test for Steroids

A quantity of (9 ml) of ethanol was added to 1 g of the extracts, refluxed for few minutes and filtered. To 1 ml of the extracts filtrates, 2 ml of chloroform and 1ml of sulphuric acid was added; formation of reddish brown ring at interface indicated the presence of steroids.

Test for Phenols

10 mls of methanol was added to 0.2 g of the extract in a test tube and shaken thoroughly. The mixture was left to stand for 5 minutes before filtered with Whatman filter paper No 1. To (1 ml) of the filtrate, 2 ml of distilled water was added followed by 0.5 ml of sodium carbonate and Folin Ciocalteau's reagent (0.5 ml), blue/green colour was formed indicating the presence of phenols.

Quantitative Phytochemical Determination

Steroids

One gram (1 g) of the extract was macerated with 20 ml of ethanol. Two milliters (2 ml) of chromagen solution was added to 2 ml of the filtrate and allowed to stand for 30 minutes. Absorbance was read at 550 nm. A standard was made following the same procedure at different concentrations using steroid hormone, a standard curve of absorbance vs concentration was plotted and the concentration of steroid in the extract extrapolated from the standard curve.

Saponins

The extracts (1 g) each was macerated with 10 ml of petroleum ether and decanted into a beaker. Another 10ml of petroleum ether was added into the beaker and the filtrate heated to evaporate into dryness. The residues was dissolved in 6 ml of ethanol. The solutions (2 ml) was then put into test tubes and 2 ml of chromagen solution added. The mixtures was allowed to stand for 30 minutes and absorbance was read at 550 nm. A standard was made following the same procedure at different concentrations using ursolic acid. A standard curve of absorbance vs concentration was plotted and the concentration of saponin in the extracts extrapolated from the standard curve.

Alkaloids

An aliquot of (0.5 g) of the extract was dissolved in 96% ethanol and 20% H_2SO_4 and filtered, the filtrate (1 ml) was added to 5 ml of 60% tetraoxosulphate (VI) acid and allowed to stand for 3 hours after which reading was taken spectrophometrically at 565 nm wavelength. A standard was made following the same procedure at different concentrations using caffeine, a standard curve of absorbance vs concentration plotted and the concentration of alkaloids in the extracts extrapolated from the standard curve.

Glycosides

The extracts (1 g) each was macerated with 50 ml of distilled water and filtered. To the filtrates (1 ml), 4 ml of pirate solution was added; the mixture was boiled for 5 minutes and allowed to cool. Absorbance was read at 490 nm. A standard was made following the same procedure at different concentrations using digitoxin, a standard curve of absorbance vs concentration plotted and the concentration of glycosides in the extracts was extrapolated from the standard curve.

Tannins

One gram (1 g) of the extract was macerated with 50 ml of methanol and filtered. To the filtrate (5 ml), 0.3 ml of 0.1N ferric chloride in 0.1N HCl and 0.3 ml of 0.0008M of potassium ferricyanide was added, mixed and the absorbance read at 720 nm. A standard was made following the same procedure at different concentrations using tannic acid as standard. A standard curve of absorbance vs concentration was plotted and the concentration of tannins extrapolated from the standard curve.

Flavonoids

The extracts (1 g) each was macerated with 20 ml of ethylacetate for 5 min and filtered. To (5 ml) filtrate was added 5 ml of dilute ammonium, shaken for 5 min, the upper layer was collected and the absorbance read at 490 nm. A standard was made following the same procedure at different concentrations using quercetin as standard. A standard curve of absorbance vs concentration was plotted and the concentration of flavonoid in the extract extrapolated from the standard curve.

Phenol

The extract (1 g) was weighed out and dissolved in 20 ml of 80% ethanol and filtered. To 5 ml of the filtrate was added 0.5 ml of Folin-Ciocalteu's reagent and allowed to stand for 3 minutes. This was followed by the addition of 2 ml of 20% sodium carbonate (Na_2CO_3). The absorbance of the mixture was read at 650 nm. A standard was made following the same procedure at different concentrations of gallic acid. A standard curve of absorbance against concentration was plotted and the concentration of phenol extrapolated from the standard curve.

RESULTS

The results of the phytochemical analysis of the leaf of touch and dye plant is presented in the tables below.

Table .1: Qualitative phytochemical composition

Phytochemicals	
Alkaloids	+
Glycoside	+
Saponins	++
Tanins	+
Flavonoids	+
Steroids	+
Phenols	+

+ = Moderately present

++ = Abundantly present

Table 2: Quantitative phytochemical composition

Phytochemicals	Quantities
Alkaloids	1021 ± 13.2 mg/dl
Glycoside	0.01 ± 0.4 mg/100g
Saponins	1.6 ± 0.1 mg/100g
Tanins	14.34 ± 0.4 mg/100g
Flavonoids	243 ± 41 mg/dl

Steroids	1.01 ± 0.0 mg/100g
Phenols	674.23 ± 00 mg/100g

Discussion

Phytochemical analysis of leaf of touch and dye plant (Mimisa pigra) are presented in Table 1. A phytochemical analysis is very useful in the evaluation of some active biological compound of some medicinal plants. The medicinal properties of the plant could be attributed to the presence of bioactive compounds in ethanol extracts under study. The extracts have shown the presence of all the seven parameters analysed (flavonoid, alkaloid, tannins, phenol, steroid, glycoside and saponins.

The quantitative phytochemical analysis showed leaf of touch and dye plant (Mimisa pigra) to contain Alkaloids (1021 ± 13.2 mg/dl), as the highest phytochemical, followed by Phenol (674.23 ± 00 mg/100g), flavonoid (243 ± 41 mg/dl), Tannin (14.34 ± 0.4 mg/100g), saponin (1.6 ± 0.1 mg/100g), steroid (1.01 ± 0.0 mg/100g), and glycoside (0.01 ± 0.4 mg/100g) is the least. The high concentration of alkaloid and phenol in the leaf showed they possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Egba *et al*., 2012). Saponins have the property of precipitating and coagulating red blood. These plants are used to stop bleeding and in treating wounds (Okwu, 2011). They exhibit foaming properties and cell membrane- permeabilizing properties. Their soapy character is due to their surfactant properties (Noudeh *et al.*, 2010). Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007). Tannins are potential metal ion chelator, proton precipitating agents and biological antioxidant. Ellagitannins have free radical scavenging activity. Phytochemicals are as antimicrobial compounds, have made great contribution for quick and effective management of plant disease and microbial contamination in several agricultural conditions. The results

This finding conforms to the report of Deshpande, (2007) in which similar constituents was found to exhibits antiprotozoal and antibacterial activities. Flavonoid has also been reported to have greater potential benefit to human Health (Jouad *et al*., 2001). Imaran *et al*. (2010) studied that phytochemical analysis of watermelon fruits by using different solvent such as Petroleum ethar, chloroform, methanol show the presence of triterpenes, glycosides and fatty acids. Other phytochemicals studied in this analysis were absent in all extract of leaves.

Flavonoids**:** have protective effects including anti-inflammatory, anti-oxidant, antiviral, and anti-carcinogenic properties. They are generally found in a variety of foods, such as oranges, tangerines, berries, apples and onions *Citrullus lanatus* can be a good dietary source of flavonoids.

Phytochemicals such as saponins, steroids, flavonoids and alkaloids have been shown to have anti-inflammatory effects. Although terpenoids were absent, the presence of alkaloids, flavonoids and saponins in therefore supports the use of touch and dye plant (Mimisa pigra) in treatments of some diseases such as stomach problems, birth defect etc. Medically the presence of these phytochemicals especially the phenols and flavonoids explains the use of touch and dye plant (Mimisa pigra) leaf extract in ethnomedicine for the management of various ailments.

Conclusion

The leaf of touch and dye plant (Mimisa pigra) contain phytochemicals like Flavonoid, Alkaloids, Tannin, Glycosides, Saponin, and steroids. These plants containing all these phytochemicals show that is highly medicinal and is good for human consumption. It is hoped that these information on the phytochemical constituents and their ethno-medicinal properties would be useful in agriculture as food supplement and for evaluation of the plant in medicine which may lead to drug discovery.

Recommendations

1. Therefore, it is of great interest to carry out a further screening of these plant extracts in order to reveal all their active ingredients by isolation and characterization of their phytochemical constituents and carry out further pharmacological evaluations.

2. The leaf of touch and dye plant (Mimisa pigra) are recommended for use in herbal medicine since it contains bioactive chemical.

3. In view of the immense content of phytochemicals in the leaves, I recommended that more research be carried out for appropriate application in pharmaceutical industries.

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