

PRELIMINARY PHYTOCHEMICAL SCREENING OF BIOACTIVE CHEMICALS IN SUNFLOWER (*Tithonia diversifolia*) ROOTS

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Abstract - Plants are the most available sources of nutrients and phytochemicals; they have also played an important role in the development of drugs and treatment of various ailments in many countries. Therefore, this study was carried out to ascertain the preliminary phytochemical screening of bioactive chemicals in wild sunflower (*Tithonia diversifolia*) root. The solvents used for extraction includes: petroleum ether, methanol and distilled water. Results revealed that tannins, flavonoids, phenols, steroids, glycosides, carbohydrates and protein were present in the aqueous extract; only saponins were not detected in the sample. Methanol and petroleum ether follow similar pattern as tannins, flavonoids, phenols, steroids, glycosides, saponins carbohydrates and protein were all present in the sample. It was concluded that the extract contained several bioactive chemicals which could confer it the ability to have therapeutic or pharmacological effects on human and animals.

Key words: *Tithonia diversifolia*, phytochemicals, nutrients, chemicals.

1. INTRODUCTION

Tithonia diversifolia (Hemsley) A. Gray is a representative of the sunflower family (Asteraceae) and it is popularly called Tree Marigold, Mexican Sunflower, Japanese Sunflower (Kawini et al., 2017). The plant is about 2.5 m high, bushy and much branched. It reproduces from seeds and through vegetative re-growth of the basal stem when the plant is cut. The stem is quadrangular, spirally-ridged, pubescent below and glabrous above. The leaves are simple, alternate, lobed and of about 5-15 cm long and 3.5-6 cm broad. It is dark green, toothed and wedged shaped at the base

(Akobundu and Agyakwa, 1997). Flower heads are yellow, large, daisy-like, on peduncles 7 to 30 centimeters long. Petals are 7 to 15, bright yellow, 4 to 7 centimeters long and 9 to 16 millimeters wide, with three small teeth at the tips (Oyewole et al., 2008; Chukwuka et al., 2007). Center of the flower heads have about 80 to 120 tiny tubular florets surrounded by several rows of green bracts. Seeds are 4 to 8 millimeters long and topped with a ring of scales and two awns, blackish in color, and somewhat four-angled (Umar et al., 2015; Tagne et al., 2018).

Phytochemical screening of flowers yielded phenolic compounds (tannins, flavonoids, and total phenols), with no alkaloids and saponins (Robson et al., 2014; Wahyuningsih et al., 2015). Proximate analysis of stems yielded moisture 20.6%, total ash 6.55%, acid insoluble ash 0.33%, and sulphated ash 14.0%. Nutrition analysis yielded protein 9.62%, fat 4.21%, fiber 15.82%, and carbohydrate 70.35% (Essiett and Akpan, 2013). Scientific studies have suggested antimicrobial, analgesic, anti-inflammatory, antidiabetic, chemopreventive, hepatoprotective, repellent, antimalarial, anti-diarrheal, antiemetic, radical scavenging, phytoremediative, biolarvicidal properties of the plant parts (Olutobi and Olasupo, 2012) and plant (stem, leaf and roots) has been traditionally used for the treatment of snake bites, malaria, diabetes, sore throat, wounds and constipation (Martinez et al., 2008; Doughari, 2012).

The objective of this study was to do a preliminary phytochemical screening of bioactive chemicals in sunflower (*Tithonia diversifolia*) roots.

2. MATERIALS AND METHODS

Experimental Site

The study was carried out at the department of biochemistry, Kano State University of Science and Technology, Wudil, Kano State.

Sample collection and processing

Fresh roots of wild sunflower (*Tithonia diversifolia*) were harvested from the institutions teaching and research farm. The species were identified and authenticated at the herbarium department of crop science, Kano State University of Science and Tech. Nigeria, where the voucher specimen was prepared and deposited. Samples were washed with running tap water, air dried for 12 days and grounded into powder using a mortar and pestle and stored in an air tight well labeled container. 100 g of the grounded sample was dissolved in 500 mL each of petroleum ether, methanol and distilled water for 48 hours, the samples were stored in the refrigerator and the extracts were filtered separately with Whatman filter paper No.1 to obtain filtrates which was subjected to further analysis.

Preliminary screening of bioactive chemicals

The screening covered the following parameters:

Tannins (Ferric chloride and Lead acetate test), flavonoids (Pew's, Shinoda and NaOH test), alkaloids (Iodine, Wanger's and Dragendorff's test), Phenols (Ellagic acid test), Saponins (foam test), Sterols (Salkowski test), Glycosides (Keller-Killani and conc. H₂SO₄), carbohydrates (Molisch's and Seliwanoff's test) and protein (Biuret test)

Procedures

Test for Tannins

Ferric chloride test

2 ml of test solution followed by addition of few drops of 5 % ferric chloride solution. The formation of a blue colour indicates the presence of hydrolysable tannins (Kokate, 2005; Harbone, 1998).

Lead acetate test

2 ml of test solution in a test tube, a few drops of 10 % lead acetate solution was added, a formation of a yellow or red precipitate indicated the presence of condensed tannins (Tease and Evans, 1985).

Test for Flavonoids

Pew's test

Zinc powder was added to 2 ml of extract in a test tube followed by drop wise addition of conc. HCl. The

formation of purple red or cherry colour indicates the presence of flavonoids (Peach and Tracey, 1956).

Shinoda test

2 ml of the extract was added in the test tube with few fragments of magnesium metal followed by drop wise addition of conc. HCl. Formation of magentia colour indicates the presence of flavonoids (Kokate et al., 2001).

NaOH test

2-3 ml of the extract was put in a test tube and few drops of sodium hydroxide solution were added. Formation of intense yellow colour which becomes colourless on addition of few drops of dilute HCl indicates the presence of flavonoids (Khandewal, 2008).

Test for alkaloids

Iodine test

3 ml of extract was poured into a test tube, few drops of dilute iodine solution was added. Formation of a blue colour which disappears on boiling and reappears on cooling indicates the presence of alkaloids (Khandewal, 2008).

Wagner's test

2-3 ml of the extract was put in a test tube followed by few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids (Kokate et al., 2001).

Dragendorff's test

2-3 ml of the extract was put in a test tube, a few drops of Dragendorff's reagent. The formation of an orange brown precipitate indicates the presence of alkaloids (Kokate et al., 2001).

Test for phenol

Ellagic acid test

3 ml of the extract was treated with few drops of 5 % (w/v) glacial acetic acid and 5 % (w/v) NaNO₂ solution in a test tube. Formation of a muddy or niger brown coloration indicates the presence of phenol (Gibbs, 1974).

Test for Saponins

Foam test

2 ml of the extract was diluted with 20 ml of distilled water; it was shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponin (Kokate et al., 2001).

Test for Sterol

Salkowski's test

2 ml of the extract with 2 ml of chloroform and 2 ml conc. H₂SO₄ were added in a test tube and shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols (Kokate et al., 2001).

Test for glycosides

Keller-Kiliani test

3 ml of the extract and glacial acetic acid were put in a test tube followed by one drop of FeCl₃ and conc. H₂SO₄. Reddish brown appears at the junction of the two liquid layers and upper layer bluish green colour indicates the presence of glycosides (Kokate et al., 2001).

Concentrated H₂SO₄ test

5 ml of the test material was mixed with 2 ml glacial acetic acid in a test tube, followed by the addition of one drop of 5 % FeCl₃ and conc. H₂SO₄. Formation of a brown ring indicates the presence of glycosides (Kokate et al., 2001).

Test for carbohydrates

Molisch's test

2 ml of extract in a test tube, add a few drops of 1 % alpha-naphthol followed by 2 ml of conc. H₂SO₄. A reddish violet or purple ring indicates the presence of carbohydrates (Harbone, 1998).

Seliwanoff's test

1 ml of extract in a test tube was added to 3 ml of Seliwanoff's reagent and it was heated on water bath for 10 minutes and cooled followed by 1 % nitrate solution. A formation of red colour confirmed the presence of carbohydrates (Harbone, 1998; Kokate et al., 2001).

Test for protein

Biuret test

3 ml of extract was placed in a test tube followed with the additions 5 drops of melons reagent and 2 ml of 10 % NaOH and mixed thoroughly. Purple or violet colour is an indication of the presence of protein (Harbone, 1998).

RESULTS

Test	Aqueous	Methanol	Petroleum ether
(1) Test for tannins			
Ferric chloride test	+ve	+ve	+ve
Lead acetate test	+ve	+ve	+ve
(2) Test for flavonoids			
Pew's test	+ve	+ve	+ve
Shinoda test	-ve	+ve	+ve
NaOH test	-ve	+ve	+ve
(3) Test for alkaloids			
Iodine test	-ve	+ve	-+ve
Wagner's test	-ve	+ve	+ve
Dragendorff's test	-ve	+ve	+ve
(4) Test for phenols			
Ellagic acid test	+ve	+ve	+ve
(5) Test for saponins			
Foam test	-ve	-ve	-ve
(6) Test for sterols			
Salkowski's test	+ve	+ve	+ve
(7) Test for glycosides			
Keller-Kiliani test	+ve	+ve	+ve
Conc. H ₂ SO ₄ test	+ve	+ve	+ve
(8) Test for carbohydrates			
Molisch's test	+ve	+ve	+ve

Seliwanoff's test	+ve	+ve	+ve
(9) Test for protein			
Biuret test	+ve	+ve	+ve

+ve : positive; -ve : negative

DISCUSSION

Table 1 revealed the preliminary screening of the bioactive chemicals in Wild sunflower (*Tithonia diversifolia*) roots. The result revealed that the sample contains several phytochemicals (bioactive chemicals) such as: protein, carbohydrates, tannins, phenols, steroids, glycosides, saponins, alkaloids and flavonoids. Petroleum ether, methanol and distilled water (aqueous) were used for the extraction process, variations were observed in the extracts. The aqueous extract revealed the presence tannins, flavonoids, phenols, steroids, glycosides, carbohydrates and protein. Saponins are the only compounds not found in wild sunflower root extract but contrary to the results observed in petroleum ether and methanol solvents which follow similar pattern, their results showed that the sample contained tannins, saponins, flavonoids, phenols, steroids, glycosides, carbohydrates and protein.

Phytochemicals are primary (chlorophyll, protein and carbohydrates) and secondary compounds (alkaloids, flavonoids, tannins, terpenoids, phenolic compounds etc.) that occur naturally in plants that help them perform multiple biological activities such as: anti-inflammatory, antioxidant, antiviral, antifungal, anti-allergic, antispasmodic, chemoprotective and hypolipidemic properties (Takeda et al., 2008; Varsha et al., 2013; Alagbe, 2019; Musa et al., 2020). Medicinal plants are also reservoirs of biologically active compounds with therapeutic properties that are used for the treatment of various ailments (Shittu and Alagbe, 2020; Dilfuza et al., 2015; Shittu et al., 2020). The variations in the phytochemicals in wild sunflower root could be attributed to the method or extraction process,

species differences, age, location, soil type and harvesting regimen (Omolere and Alagbe, 2020; Alagbe et al., 2020). Alkaloids exhibit various pharmacological activities such as: antimicrobial, antispasmodic, analgesic and antibacterial activities (Olafadehan et al., 2020; Edeoga et al., 2006; Faizi et al., 2008). Saponins are generally known for their antibacterial and antifungal properties (Olaleye, 1997; Cheeke, 2000; Alagbe and Soares, 2018). Phenols are strong antioxidants that scavenge free radicals (Hollman, 2001). Flavonoids possess anti-inflammatory, anti-allergic, anti-thrombotic and vasoprotective properties (Chen et al., 2000; Saleem et al., 2005). Tannins are known to possess antibacterial and antiviral activity (Adisa et al., 2010; Olafadehan et al., 2020; Oluwafemi et al., 2020). Proteins play a central role in cell growth and strengthening the immune system (Akubugwo et al., 2007). Carbohydrates serve as a source of energy to animals and they are produced by plants during photosynthesis (Vasudevan and Steekumari, 2007; Olatunji et al., 2016).

CONCLUSION

The extraction of wild sunflower roots using different solvent have shown that the plant is loaded with various bioactive chemicals or phytochemicals which are capable of performing physiological action on the body of animals. Each medicinal plant has its own nutrient composition besides the pharmacological effects and it can be used for the treatment of various ailments especially among rural dwellers.

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