AJBCPS African Journal of Biological, Chemical and Physical Sciences

Vol 1, Issue 1, 2022

ISSN: 2790-9522 (Print) | 2790-9530 (Online)
Website: https://journals.jozacpublishers.com/ajbcps/index

OPEN ACCESS

Evaluation of antioxidant activity, phytochemical screening, FTIR characterisation and nutritional values of *Passiflora Foetida* methanol extract

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Received: March 19, 2022 | **Accepted:** April 30, 2022 | **Published:** May 06, 2022

Abstract

Hysteria, asthma, and skin illnesses with inflammation are all treated by *Passiflora foetida*, a wild species of the Passifloraceae family. The purpose of this work is to determine the phytochemical contents, antioxidant capabilities, and FTIR analysis of *Passiflora foetida* crude methanol extract. Standard laboratory methods were used to conduct the phytochemical analysis. The antioxidant activity of the methanol extract was measured using an UltraViolet-Visible Spectrophotometer at 517nm against 2, 2-diphenyl-1-picryhydroxyl (DPPH). In addition, an FTIR characterization study was performed. The sample, according to the phytochemical screening analysis, contains tannin, saponins, flavonoids, alkaloids, and phenols. At 300µg/mL concentration, the highest antioxidant activity was recorded (73.36 percent), whereas, at 1000µg/mL concentration, the lowest activity (60.31 percent) was obtained. FTIR spectrum bands of 2852–2922 cm^{-1} -CH- group, 1507 – 1715 cm^{-1} band of C-C aromatic group, 1173 – 1362cm^{-1} band of C-O aromatic and ester groups are notable in the spectrum. The results of this study revealed that Passiflora foetida may be a promising plant to be exploited in drug discovery.

Keywords: Antioxidants, FTIR analysis, *Passiflora foetida*, Saponin, Tannin

1. Introduction

Passiflora foetida is a class of passion flowers belonging to the Passifloraceae family. This plant can be found all over the world in tropical regions. It is a creeping plant that bears eatable fruit, as it is in the genus Passiflora. In Latin, the meaning of the word foetida is stinking, alluding to the unpleasant odour produced when the leaves spoil. For a long time, the Passiflora species has been utilized in traditional medicine (Miroddi et al., 2013). Passiflora is useful in remediating a variety of health conditions, including insomnia, female hysteria, and seizures. Wounds and bruises can also be treated with it. It's been a long-time therapeutic aid to women with menopausal symptoms like hot flashes and insomnia. Convulsive diseases have been demonstrated to benefit from the use of

Passiflora. According to Dr. Vikram Chauhan (https://www.planetayurveda.com), the plant has been shown to be effective in treating *Clostridium tetani* (tetanus) in both humans and horses. All organs of *P. foetida,* including the leaf, root, and stem, have been discovered to contain medicinal activities, making it a prospective target for new drug development (CABI, 2013).

2. Literature review

Passiflora foetida is one of about 300 high pharmaceutical plants found in Cote d'Ivoire, where about 5000 species have been identified, according to (Chandel et al., 1980). *P. foetida* has been used in the treatment of a variety of human ailments and disorders. According to Yuldasheva et al. (2004), it has a substantial anxiolytic and sedative effect (2004). This herb is employed in treatment of insomnia, epilepsy, *Clostridium tetani*, and muscle spasms in homeopathy (Woode et al., 2009). Newcastle disease in chickens is treated with various formulations of *P. foetida* fruits, leaves, stems, and seeds (Deginani, 1998). Chronic pain, cough, asthma, insomnia, hysteria, biliousness, and digestive disorders, particularly dyspepsia, can all be treated using the organs of *P. foetida* (Da Costa Sacco et al., 1980). Fungicidal activities *of P.foetida* (Patil et al., 2015) are reported. *P. foetida* leaf extracts have antibacterial activity on human pathogens such as *Pseudomonas putida, Vibrio cholera, Shigella flexneri,* and *Streptococcus pyogenes* (Hoffmann et al., 2003). In ulcer rat stomach tissue, *P. foetida*'s anti-ulcer and antioxidant activities were established, and the antiulcerogenic action was linked to antioxidant activity. Hysteria and sleeplessness are treated with infusions of the leaves in Nigeria, and aerial sections are used to cure kteria, hepatitis, constipation, esophagitis, and discomfort in Benin (Santosh et al., 2011). According to Yuldasheua et al. (2005), the Passifloraceae family has only a few articles on proximal and cytotoxic assessments. In addition, *P. foetida* species has been badly endangered due to the therapeutic efficacy of the plant and the huge quantity of unrestrained harvest and extraction to meet increasing demands by the therapeutic industry, along with limited cultivation and poor replanting attempts. (Wood et al., 2009). This study looks at the antioxidant properties, phytochemical content, nutritional benefits, and FTIR characterization of functional groups in *P.foetida*.

Figure 1: Image of *Passiflora foetida*

3. Research methodology

The fresh and healthy aerial parts of *Passiflora foetida* were harvested around the Chemistry Advanced Research Centre (CARC) of Sheda Science and Technology Complex (SHESTCO) in FCT, Abuja. The

harvested plant sample was screened and separated from foreign materials; air dried and pulverized using a mechanical hammer mill. The powdered sample was stored in a plastic bag till further usage. 250g of the powdered sample was macerated with 750 ml methanol for 72 hours. The extract was filtered using filter paper to separate the marc from the extract, and the solvent was evaporated using rotary evaporator.

3.1. Phytochemical screening

Phytochemical screening was carried out using standard procedures as described below.

- **Test for Phenols:** The phenol test was carried out using the Sofowora (1993) method. In a test tube, 2 mL of the extract was obtained and deposited. 2mL ferric chloride solution was added after that. The presence of phenols was indicated by a deep bluish-green solution.
- **Test for Terpenoids:** Edeoga et al. (2005) describe the Salkowski test for terpenoids. A 5mL extract was treated with 2mL of chloroform. The color turned out to be blue. After 3mL of concentrated H_2SO_4 was added to the mixture, the formation of a reddish-brown color at the junction was confirmed as a positive test for terpenoids.
- **Detection of Saponins:** This test was performed according to the Edeoga et al. (2005) protocol. 2g of powdered material was boiled in 20mL of distilled water using a water bath for heating. Filtration was done after cooling. Then, 5 mL of distilled water was added to the filtrate and aggressively shaken to produce stable foam. An emulsion developed when three drops of olive oil were added to the foam and violently shaken, confirming the presence of saponins.
- **Flavonoids test:** The flavonoids test was carried out using Harborne's approach (1973). For 1 g of powdered material, it was boiled for 5 minutes at temperature range of 40–50°C, with 10 mL of ethyl acetate. A 1 mL diluted ammonia solution was added to the filtrate. Flavonoids were visible as a golden yellow color.
- **The test for alkaloids:** Harborne's (1973) protocols were followed for the alkaloids test. To 1 gram of powdered material, 5 mL of methanol and 5 mL of 2M hydrochloric acid were used to extract 5 mL of the sample. Meyer's and Wagner's reagents were used to treat the filtrate. The turbidity of the samples was used to determine if they were positive.
- **Detection of Tannins:** The Tannins test was performed using the Kumar et al. (2007) approach (2007). To 2-3 mL of methanol extract, a 10% alcoholic solution of ferric chloride was added. The formation of a dark blue colour confirms the presence of tannins.
- **Detection of Steroids:** To identify the steroids, the approach outlined by Edeoga et al., (2005) was used. The colour of the 1mL extract solution changed from blue to dark green when 2mL acetic anhydride and 2mL concentrated sulfuric acid were added, suggesting the presence of steroids.
- **FT-IR Spectroscopy:** A Thermo Fisher Scientific (Nicolet IS 5), USA Fourier Transform Infrared (FTIR) spectrophotometer was used to scan the methanol extract of *P. foetida* between 400 and 4000 cm^{-1} wavelengths. The characteristic functional groups of the plant extract are revealed by FTIR spectra.

3.2. Antioxidant capacity measurement

A modified Brand-Williams approach was used to measure antioxidant activity [Brand-Williams et al, 1995]. In methanol, the reagent, (DPPH), 2, 2-diphenyl-1-picryhydroxyl, is scavenged. The level of discoloration from purple to yellow shows the ability of the extract to scavenge free radicals provided by the DPPH. At varied concentrations of extract (100, 300, 500, 700, and 1000µg/mL), the absorbance was measured at 517nm against a blank solution. Ascorbic acid was utilized as a standard positive control at the same concentrations. The ability of extract to scavenge radicals is calculated using the following formula:

$$\text{Percent inhibition} = \frac{B - S}{B} \times \frac{100}{1}$$

Where, B = Absorbance of blank solution, S = Absorbance of sample

The antioxidant ability of the extract was determined by the IC50 concentration.

3.3. Proximate/nutrition analysis

The nutritional values of *P. foetida* were determined using the AOAC standard method [AOAC, 1990] for proximate analysis. Moisture%, ash%, crude fat%, crude fiber%, crude protein%, and carbohydrate% are all parameters for determination. The mean and standard deviation of each parameter were computed after three tests.

4. Results and discussions

Table 1: Qualitative Phytochemical

Tannins	+
Flavonoids	+
Saponins	+
Alkaloids	+
Glycosides	+
Terpenoids	+
Phenols	+

Key: + means Present/Detected

Table 2: Antioxidant activity of methanolic extract of *P.foetida* and ascorbic acid

Conc (µg/mL)	%AA of *P. foetida*	% AA of Vit C
100	61.63±0.22	60.38±0.31
300	73.36±0.34	72.50±0.28
500	69.42±0.32	77.69±0.26
700	65.47±0.27	77.88±0.36
1000	60.31±0.30	81.92±0.26

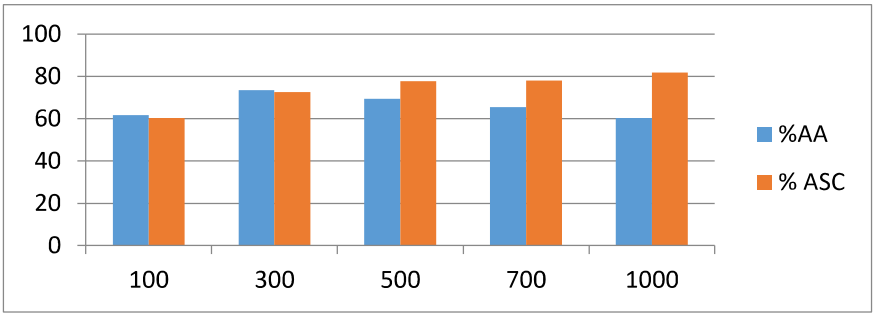

Figure 2: Comparison of antioxidant capacities of *P.foetida* and ascorbic acid

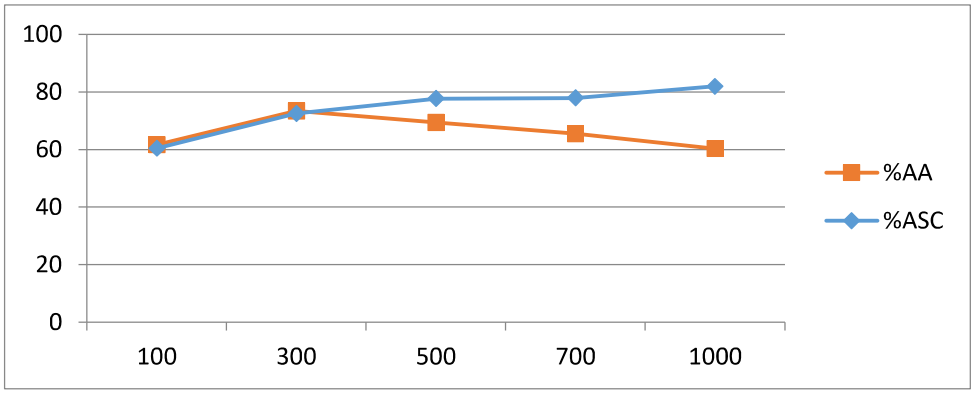

Figure 3: Nonlinear regression of % antioxidant vs. concentration (µg/mL) of *P.foetida* (AA) and ascorbic acid (ASC)

Table 3: Proximate result for *P.foetida*

Composition	% weight
Moisture	7.76±0.07
Ash	10.49±0.14
Crude Lipid	18.36±0.15
Crude Fibre	28.45±0.04
Crude protein	BDL
Carbohydrate	34.94±0.11

NOTE: BDL means below detection limit

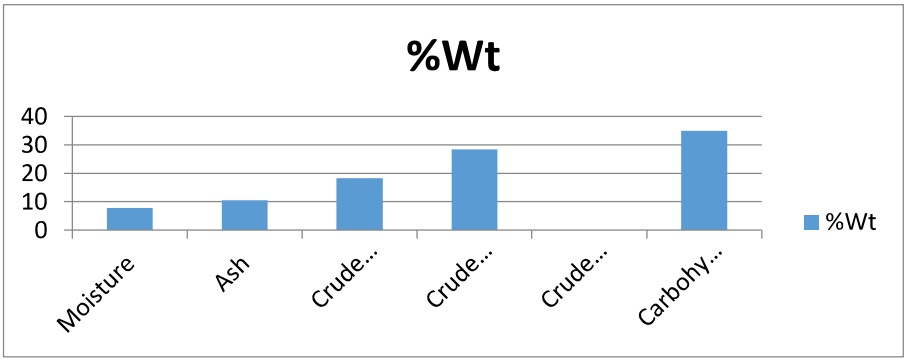

Figure 4: Weight % composition of nutrients in *P.foetida*

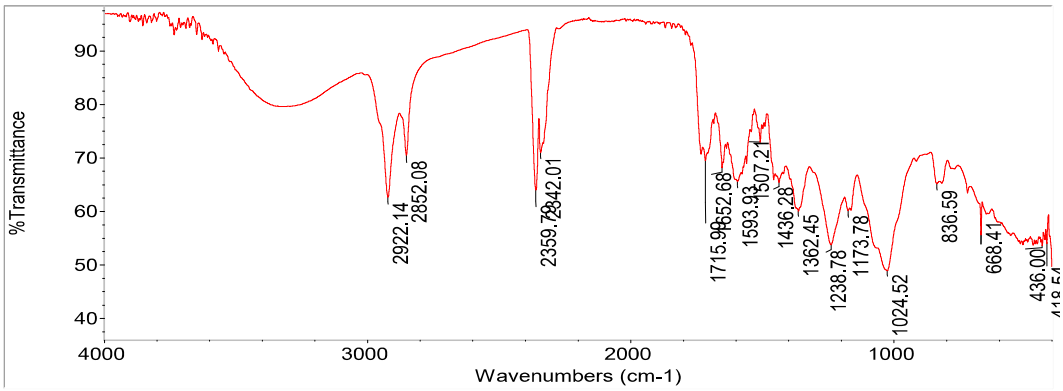

Figure 5: FTIR Spectrum of *P. foetida*

Table 4: FTIR Absorption bands

FTIR Absorption band (cm^{-1})	functional group
2922.14, 2852.08	-CH-
1715.99, 1652.68, 1593.93, 1507.21	C-C aromatic
1362.45, 1238.78, 1173.78	C-O aromatic and ester
1024.52	C-O ester
836.59, 668.41, 436.60	C-C aromatic ring

4.1. Discussion

P. foetida contains alkaloids, saponins, flavonoids, phenols, tannins, steroids, glycosides, and terpenes, according to the results of the qualitative phytochemical investigation (Tables 1). This is a sign of its therapeutic potential. Because of their reducing capabilities, plants' phenolics act as antioxidants by serving as reducing agents, donors of hydrogen, and free radical scavengers. (Javanraedi et al., 2003). Flavonoids have a significant role as antioxidants. Antimicrobial, antiallergic, anti-inflammatory, and anticancer compounds are also found in them. They play critical functions in reproduction and growth. Flavonoids also protect plants from pathogenic microorganisms and predators (Rice-Evans et al., 1996; Enechi et al., 2016). Tannins are known for their immune-stimulating properties. Tannins have an important part in wound healing. Tannins can also act as a main antioxidant or scavenger of free radicals (Polteraitet et al., 1977). The presence of phenolic components or polyphenols, flavonoids, or tannins in *P.foetida* extract could explain the antioxidant activity found. This agrees with the result of (Nahak et al., 2010). Alkaloids are stimulants, pain relievers, and sedatives. Because of their stimulatory actions, alkaloids can be dangerous when consumed in excessive doses, causing excitation associated with cell and nerve diseases (Jisika et al., 2010; Obochi et al., 2006).

Saponins are a type of phytochemical that is very important. They're triterpenoid or steroidal glycosides with antiallergic, cytotoxic, antitumor, antiviral, and antifungal properties (Musa et al., 2011). Saponins are utilized in animal vaccines because they help the immune system respond better. Many saponins are beneficial in intracellular histochemistry labeling, which allows antibodies to reach intracellular protein molecules. The DPPH scavenging activity on the methanolic extract of *P.foetida* revealed that the plant is a good source of antioxidant (Fadeyi et al, 2020). It highest activity

at a concentration of 300µg/mL is 73.36% compared with that of the ascorbic acid 72.50% at same concentration. The lowest activity (60%) is recorded at the highest concentration, which surprisingly is the highest (81.92%) for recorded activity for the positive control, ascorbic acid. The IC50 of *P.foetida* is 58.09 while that of the ascorbic acid is 41.64. Proximate analysis was done in triplicate and the standard deviation calculated. The summary of the result is in Table 3. The result indicated a high %crude fibre (28.45±0.04). This can be advantageous in feed formulation where high fibre is required. The %protein content is unusually low. It clearly suggests that it is not a good source of protein.

The %lipid is 18.36±0.15, the %ash content is 10.49±0.14, %moisture 7.76±0.07 and %carbohydrate, 34.94±0.11. Table 4 highlights the main peaks in the FTIR spectra (figure 5) and the bonds they represent. From the FTIR spectra, the following bonds are noticeable 2922.14, 2852.8 which represents the –CH- stretch of CH_3 and -CH- of CH_2 respectively. Also, C-C aromatic group at absorption bands of 1715.99, 1652.68, 1593.93, 1507.21 cm^{-1}. C-O aromatic and ester bands at 1362.45, 1238.78, 1173.78 and C-O bands at 1024.52. C-C aromatic rind at absorption bands 836.59, 668.41 and 436.60 cm^{-1}.

5. Conclusion

Results from this study on *P.foetida* showed that plant can be exploited for drug development due to its phytochemical constituents and the actioxidant activites. The aromatic functional groups in the FTIR spectra also confirm the aroma naturally produced by the plant. The plant may also be used as an ingredient in formulation of animal feed due to it rich fibre content.

6. Acknowledgments

The author wish to acknowledge the management of Sheda Science and Technology for providing the space and reagents used for this work

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