



PHYTOCHEMICAL STUDIES ON *PORTULACA OLERACEA* (*PURSLANE*) PLANT

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Abstract

Portulaca oleracea (*PO*) is globally used both as a vegetable and as a herb for medical and therapeutic purposes; hence the need to investigate its phytonutrients. The aerial parts of *PO* were harvested, air dried and powdered for this study. Chemical tests were carried out on the aqueous extract and the powdered specimen to determine the phytoconstituents using standard procedures. The presence of Alkaloid, saponin, tannin, flavonoid, cardiac glycoside, terpenoid, steroid, phobatanin, protein and starch were accessed qualitatively while flavonoid, tannin alkaloid and saponin were determined quantitatively and it was found not to contain steroid and phobatanin but containing 32% of saponin as its highest constituent with 26% alkaloid. This finding authenticates its use in drug production and other therapies, and to enhance further its usage and research- study.

Keyword: Herb, phytoconstituents, quantitative, qualitative, screening.

Introduction

Plant's profile

PO belongs to the family of Portulacaceae. It is commonly called Purslane in English language, babbajibi in Hausa language and esan omode or papasan in Yoruba language^[5]; however it is called ntiok, ntilimoke, ntiike, or idiridi in Igbo language.

Its usage and benefits

Medicinal plants and herbs have long been used in treating diseases and illnesses^[19]. Herbs are powerhouses of nutrition and if it is used wisely and regularly, it can replace costly pills and supplements, and even some drugs^[34]. *PO* is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea'^[31].

P. oleracea is very important because of its special medical function and all its therapeutic values are attributed to the presence of many biologically active compounds which include flavonoids (Apigenin, kaempferol, quercetin, luteolin, myricetin, genistein, and genistin), Alkaloids, Coumarins, anthraquinone glycoside, cardiac glycoside, and high content of ω -3 fatty acids^[42]. The *P. oleracea* was a rich source of omega-3- fatty acids, which was important in preventing heart attacks and strengthening the immune system^[44]. Several biological properties have been attributed to *P. oleracea*: antiseptic, antispasmodic, diuretic, vermifuge^[47], anti-scorbutic, antibacterial, wound-healing^[31], analgesic, anti-inflammatory activities and skeletal muscle relaxant, bronchodilator, anti-ascorbic, antipyretic, anti-asthma, and anti-tussive effect^[23]. *P. oleracea* contains more omega-3 fatty acids (particularly alpha-linolenic acid) than any other leafy vegetable plant. It also contains vitamins A, C and E as well as dietary minerals such as calcium, potassium, magnesium and iron, pigments, betacyanins with potent antioxidants property^[16]. Other chemical constituents reported are oxalate, alkaloids, flavonoids and cardiac glycosides^[32]. The plant has been examined for its anti-inflammatory, analgesic and antifungal activities both in *invitro* and *invivo* studies^[40]. The plant is antibacterial, antiscorbutic, depurative, diuretic and febrifuge^[4,7,20,28,30]. The leaves are a rich source of omega-3 fatty acids, which is thought to be important in preventing heart attacks and strengthening the immune system^[4]. The fresh juice is used in the treatment of strangury, coughs, sores etc^[7, 20, 28, 30]. The leaves are poulticed and applied to burns^[18], both the leaves and the plant juice are particularly effective in the treatment of skin diseases and insect stings^[4,7]. A tea made from the leaves is used in the treatment of stomach aches and headaches; the leaf juice is applied to earaches, it is also said to alleviate caterpillar stings^[18]. In Nigeria the plant is used as a diuretic. The bruised leaves are used in external application for erysipelas, treatment of burns and are applied topically to swellings^[38].

Materials and Method

This study was conducted in the Department of Pharmacognosy and traditional medicine, College of Pharmacy, Nnamdi Azikiwe University Agulu campus and lasted for about one month

Plant Collection

Fresh aerial parts of *PO* plant was harvested from the surroundings of Nnamdi Azikiwe University Awka and authenticated by Department of Pharmacognosy and Traditional Medicine, College of Pharmacy, Nnamdi Azikiwe University, Agulu Campus with reference number PCG477. The plant used for both quantitative and qualitative screening was washed, cut into smaller parts (for easy drying), shade-dried for two weeks and finely powdered with a mechanical grinder yielding 550g of powder.

Preparation of Plant Extract

The aqueous extract used in this research was prepared by soaking the powdered plant (430g) in distilled water in a ratio of 1: 9 for 24 hours and the extract filtered using whatman filter paper No. 42 producing a 16% yield of extract (69.7g). The tests were carried out using different quantities of the aqueous extract.

Qualitative Phytochemical Screening

1. Test for alkaloids – 5g of the powdered plant were placed in a test tube and 20ml methanol poured into the test tube. The mixture was allowed to boil for 2 minutes in water bath, cooled and filtered. Two drops of dragendoff's reagent (solution of potassium bismuth iodide) was added to 2ml of filtrate; two drops of wagner's reagent (solution of iodine and potassium iodide) was added to 5ml of the filtrate; two drops of Meyers reagent (potassium mercuric iodide solution) was added to another 2ml of filtrate and two drops of hager's reagent (saturated solution of picric acid) was added to a 5ml of filtrate. The alkaloids are precipitated from the above solutions above giving characteristic colours – reddish brown, reddish brown, cream and yellow respectively.
2. Test for saponin – 2g of the powdered sample was boiled in 20ml of distilled water in water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously, then observed for the formation of emulsion.
3. Test for tannin – 0.5g of the powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black colouration.
4. Test for flavonoid – 10ml of ethyl acetate was added to about 0.2g of the powdered plant material and heated on a water bath for 3 minutes. The mixture was cooled, filtered and 4ml of filtrate is shaken with 1ml of dilute ammonia solution. The layers are allowed to separate and the yellow colour in the ammoniacal layer indicates the presence of flavonoids.
5. Test for cardiac glycosides (Keller-killani test): 5ml of extract was treated with 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was underlayered with 1ml concentrated sulphuric acid. A brown ring at the interface is characteristic of cardenolides. A violet ring appears below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.
6. Terpenoids: 5ml of plant extract were mixed in 2ml of chloroform and 3ml concentrated sulphuric acid carefully added to form a layer. A reddish brown colour interface was formed to show positive results for terpenoids.
7. Test for steroids – A 9ml portion of ethanol was added to 1g of the powdered leaves. This was refluxed for a few minutes and filtered. The filtrate is concentrated to 2.5ml on a boiling water bath and 5ml of hot water was added. The mixture is allowed to stand for 1 hour and the waxy matter filtered off. The filtrate extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml of the chloroform extract in a test tube, 1ml of concentrated sulphuric acid was added to form a lower layer. A reddish brown interface shows the presence of steroids.
8. Test for phlobatannin: the presence of red precipitate when an aqueous extract of the plant was boiled with 1% aqueous hydrochloric acid showed the presence of phlobatannin.
9. Test for protein – few drops of picric acid was added to a little portion of aqueous extract of plant. A yellow precipitate indicates the presence of proteins^[45].
10. Test for starch: 0.1g of the powdered plant was mixed with a drop of iodine solution in a test tube. A blue-black coloration indicates the presence of starch.

Quantitative Determination Of Phytochemical Constituents

1. Flavonoid determination by the method of Bohrn and Kocipal-abyazan (1994)^[2]: 10g of the powdered plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42. The filtrate was later transformed into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.
2. Tannin determination by Van- burden and Robinson (1981) method^[46]: 500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 605 nm within 10mins.
3. Alkaloid determination using harbore (1973) method^[21]: 5g of the powdered sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.
4. Saponin determination^[36]: The 20g of powdered plant sample was put into a conical flask and 100cm³ of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re- extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. the aqueous layer was recovered while the ether layer was discarded. the purification process was repeated.

60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. the remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight;

The flavonoid, alkaloid and saponin content were calculated as percentage using the formula:

$$\frac{\text{Final weight obtained (in grams)}}{\text{Initial dried sample used (in grams)}} \times 100$$

The percentage tannin is calculated thus:

$$A \times (0.772 / B) \text{ where } A = \text{absorbance and } B = \text{mass of powdered sample used (in grams).}$$

Results

This study revealed the presence of medicinally active phytoconstituents in PO plant sample summarized in tables 1.0 and 2.0. The qualitative estimation saw the presence of alkaloid, saponin, tannin, flavonoid, cardiac glycoside, terpenoid, protein and starch in plant sample either in abundant or very abundant measure while there is absence of steroid and phlobatannin.

Quantitative estimation(in percentages) of the four major active constituent of PO yielded a high content of saponin (32%) and alkaloid (26%) but a low content of flavonoid (6%) and tannin (0.03%) (Table 2.0).

Table 1.0. Qualitative Screening Of PO Constituents

Phytochemical constituent	Alkaloid	Saponin	Tannin	Flavonoid	Cardiac glycoside	Terpenoid	Steroid	Phlobatannin	Protein	Starch
Quantity	++	++	+	+	+	+	-	-	++	++

Table showing the presence or absence of different phytochemicals in PO.

KEY

+	Present(trace amount)
++	Abundant
+++	Very abundant
-	absent

Table 2.0 Quantitative Screening of PO Constituents

PHYTOCONSTITUENT	QUANTITY (%)
Flavonoid	6
Tannin	0.03
Alkaloid	26
Saponin	32

A table showing the quantitative analysis of some major phytochemical constituents of PO. Table shows high content of saponin and alkaloid.

Discussion

The phytochemical constituents of *P. oleracea* are presented in Table 1.0 above. Saponin, alkaloid, tannin, flavonoid, cardiac glycoside, terpenoids, protein and starch has been observed as the active phytoconstituents of PO with saponin as the major constituent. The presence of these constituents in most plants has been reported to have proven medicinal implications^[19]. The high percentage content of saponin may be indicative of the plant's major use as an antihelminthic and antiphlogistic^[3,24]. Some plant saponins have been implicated to enhance nutrient absorption and aid in animal digestion while some have life threatening animal toxicity^[22] especially cold blooded animals; this agrees with postulation by Bown^[4] that the leaf extract remedy are not given to patients with digestive problems. Saponin also acts as surfactants and adjuvants in vaccines^[43] hence its use as an antimicrobial and immune booster^[4]. The presence of tannin in the plant shows its potential as an antiviral, antibacterial and anti-parasitic^[1,29,33] while cardiac glycoside are used in the treatment of congestive heart failure and cardiac arrhythmia. Terpenoids found in the plant is against the phytochemical findings of Iyekowa et al (2012)^[25]. Terpenoids have been implicated in antibacterial and antineoplastic functions hence the use of the plant to treat burns, skin diseases and insect stings^[4,7,18]. PO which is a routine vegetable for soups and salads^[17,20] is highly nutritious with its content of starch and protein, this suggests its potential use as growth supplement for children as complemented with its use in piggyery^[5], hence it is called pigweed. The presence of flavanoids is indicative of its potential use as an anti –allergic, anti inflammatory, anti oxidative, antimicrobial anti diarrhea and anticancer^[6,8,9,35,41,48]. Antimicrobial, anti-inflammatory and antioxidative effects of PO have been observed^[26,27,39] using rat model. In support of the findings of this present study, alkaloids have been reported to be important chemical constituents of this species^[44]. Alkaloids are known with its pharmacological use for producing analgesics, stimulants, antihypertensive, anticancer, antibacterial, anti arrhythmia, anti asthma antimalarial and recreational drugs. Iyekowa et al (2012)^[25] reported the presence of steroid in PO extract as against the result of this present study; this may be differences in species or distribution of the plant. The established reproductive function of PO^[37] may be dependent on the plant content of steroid which is a potential starting material for reproductive hormones. Phlobatannin (a condensed form of tannin) has been found absent in PO examined.

Conclusion

PO is used locally for herbal medicine and as food but yet to be fully explored. The plant has been reported as a global panacea due to its several medicinal uses. The phytoconstituents observed in this study shows the plant's potency for use in producing pharmaceutical bioactive compounds for therapeutic drugs. However further studies should be carried out on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds and determine their mechanism of action. A comparative study is also necessary to determine the variations in observed phytoconstituents based on distribution.

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