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Original Research Article

Phytochemical Analysis and Antimicrobial Studies of Leaves and Roots of *P. angulata*

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Abstract

Quantifying the bioactive compounds present in plant parts (leaves and roots). This study aimed to perform comprehensive qualitative phytochemical analysis and antimicrobial analysis of extract of leaves and roots of p.angulata. phytochemicals test where carried out on the extracts to detect the presence of phytochemicals such as phenols, flavonoids, saponins, alkaloids, tannins and terpenoids etc. Different chemical tests were employed to confirm the presence of these compounds. The color change indicates the presence of specific phytochemicals. The results obtained from the qualitative analysis revealed the presence of phenols, flavonoids, saponins, alkaloids, tannins, terpenoids, phlobatannins, anthraquinones, glycosides and steroids in both the leaves and roots part of the plant. However the results of the antimicrobial test obtained from the extracts of both leaves and roots extracts of *Physalis angulata* revealed that both the crude extracts of the leaves and roots inhibited Anti-microbial activities against *Bacillus species, Styphylococcus aureus*, Plasmodium parasites and Streptococcus species except the leaves extract of acetone which does not act against *Styphylococcus aureus*. The facts that the plant was active against laboratory isolates are also an indication that the plant parts has medicinal values and Potential therapeutic or pharmacological properties. The findings of this analysis can be further utilized for developing drugs, functional foods, or dietary supplements with specific phytochemical.

Keywords: Alkaloids, Anthraquinones, Flavonoids, Glycosides and steroids, P. angulata, Phenols, Phlobatannins, Phytochemicals, Saponins, Tannins, Terpenoids

INTRODUCTION

Physalis angulata is an annual or perennial herb which belongs to the family of Solanaceae. *P. angulata* is native to North America and South America (Cletus and Ikpefan, 2016). Several species of the genus have been extensively introduced into cultivation in many parts of the world (silva et al 2005). In India, six species of the genus physalis have been reported, inhabiting tropical and temperate regions and they are growing naturally in sunny to somewhat shaded fields, pastures, roadsides and wastelands (Nahid et al., 2008). The plant *P. angulata* is also increasing naturally in Nepal, specially monsoon season in fields, pastures, roadsides, and wastelands of Terai regions. As reported by (Cheng-peng et al., 2016) *P. angulate* parts is widely used in traditionally medicine to cure inflammatory related illness such as dermatitis, asthma, malaria and hepatitis and it is also utilized to treat the analogous sickness in Indonesia, Peru, Mexico and Brazil .(Shravan et al., 2011) reported that *P. angulata* is medicinally important plant used in traditional medicine in Nigeria as anti-pyretic, anti-diuretic and cervicitis treatment. The plant parts, an herbaceous plant possesses several pharmacological activities like immunosuppressive (Lorena et al., 2016), immuno-modulatory (Silva et al., 2014) anti- inflammatory (Choi et

al., 2003), anti-cancerous activity (Kusumaningtyas et al., 2016), anti-bacterial (Silva, 2005), anti-leishmanial, diuretic, antimycobacterial, antispasmodic, anti-coagulant and anti-hyperglycaemic activity (Soares et al., 2003, Mastuti et al., 2019, and Januario et al., 2002). In Nigeria the traditional use reported is broad, wherein entirely all parts of the plant have been used for medicinal purposes; whole plant (leaves, barks, fruits and roots) is for child-birth, diuretic, fever, gonorrhea, jaundice, liver diseases, malaria, nephritis, postpartum hemorrhage, rashes, skin sores, sleeping sickness, to prevent abortion, tumors. The fruits are recommended for infection, infertility, inflammation, postpartum infection, skin diseases. The leaves are also used for asthma, dermatitis, diuretic, ear ache, fever, gonorrhea, hemorrhage, hepatitis, infections, inflammation, liver disorders, malaria, postpartum infection, rheumatism, skin diseases, to prevent abortion, worms (schistosomiasis). The root is used for diabetes, earache, fever, hepatitis, jaundice, liver disorders, malaria and rheumatism (Lawal *et al*., 2010). However as reported by Dias, 2012 attested by Dogara et *al.,* 2023, phytochemicals are in charge of many bioactivities in plants, including antioxidant, anti-inflammatory, anti-bacterial, and anticancer properties. To this, plant extracts are excellent sources of phytochemicals and identifying and characterizing these substances requires careful qualitative and quantitative study of the extracts. While quantitative analysis creates the quantity of a particular phytochemical present in the extracts, qualitative analysis identifies other types of phytochemicals. For the purpose of identifying and screening promising plant-based compounds for use in drug discovery and nutraceutical applications, these analyses offer useful information for a number of reasons; to determine the secondary metabolites with pharmacological properties in *P. angulata,* comprehensive anatomical description and phytochemical characterizations of the leaves and roots were conducted. This work sheds light on functional aspects of the secretory structures and might contribute to improve the bioprospecting process and consequently the plant's pharmacological potential and antifungal activities. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries. The use of medicinal plants is very wide spread in many parts of the world because it is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects. This knowledge aids in understanding the underlying mechanisms and inter-actions between the extract and the microorganisms, offering insights into potential target sites and pathways involved. This will also serves as the pathway to the synthesis of drugs to cure certain ailment base on the antimicrobial studies.

EXPERIMENTAL PROCEDURES

MATERIALS

All materials used were of analytical grade and reagent were obtained from commercial sources. A weighing scale, laboratory glassware, heating mantle, Soxhlet extractor, distillation set, water bath, beaker, Buchner funnel, filter paper, Whatmann number 1 paper, volumetric flask, oven, conical flask were used during the course of this study. Nitric acid (HNO_3), concentrated sulfuric acid (H_2SO_4), glacial acetic acid, ferric chloride, lead acetate, sodium hydroxide, potassium mercuric chloride solution, distilled water are some of the chemicals used in chemical reactions.

METHODS

Sample collection and preparation

The plant part (leaf and root) were collected from the plant's native environment in Wukari Local Government Area Taraba State, Nigeria. The plant part (leaf and root) was newly harvested, transported to department of botany for identification, thereafter it was taken to the laboratory and completely rinsed with running water, and then cleaned with distilled water to aid drying in the shade, the leaves and roots were cut into small pieces into different containers and dried for two weeks to reduce the moisture content. The plant components were thoroughly ground into a fine powder using a mechanical blender after drying. The powder of the leaves and roots was then maintained in the desiccator for analysis and stored in sealed containers with the appropriate labeling for easy identification.

Sample extraction

Serial exhaustive extraction is the technique utilized for extraction as described by Wakirwa *et al*., 2013 with little modification. In order to assure effective extraction of a wide range of chemicals such as alkaloid, tannins, saponins, terpenes, etc. if present in the leaves and roots this requires serial extraction with solvents in sequence of their increasing polarity from hexane to ethanol (i.e. from non-polar to more polar). The leaf and root extracts were prepared by soaking each 200g in 400ml hexane for four days while stirring frequently to dissolve any solubilized materials. The resultant mixture was filtered through Whatman No. 1 filter paper, evaporated, and concentrated into solid extracts using a rotatory evaporator, and then stored at room temperature for an overnight period to remove any solvent. In order of increasing polarity, this procedure was maintained for other chemicals like

chloroform, ethyl acetate, acetone, and ethanol in that sequence for the two samples. The extracts were kept safe until required for analysis.

Qualitative Phytochemical Analysis

The extracts of each solvent were used to examine the presence of different phytochemical components.

Test for flavonoids

A few drops of $FeCl_3$ solution were applied to the extract (Ferric chloride test). Flavonoids are present when a blackish red color is formed.(Ferric chloride test). Flavonoids are present when a blackish red color is formed.

Test for Tannins

To 1 ml of the solvent extract, few drops of 1% $FeCl_3$ solution were added. The appearance of a blue, black, green or blue green precipitate indicated the presence of tannins.

Test for terpenoids

Salkowski's test was conducted, and 2 ml of chloroform were added to 1 ml of the solvent extract. After that, 3 ml of concentrated H_2SO_4 was carefully added, forming a layer. The presence of terpenoids was revealed by the interface's reddish-brown coloring.

Test for phlobatannins

The mixture was heated after 2 ml of aqueous extract and 2 ml of 1 % HCl were added. Phlobatannins were proven to exist by the red precipitates

Test for anthraquinones

In order to perform the Borntrager's test, a small amount of the extract was shaken vigorously with 10 ml of benzene and then filtered. The filtrate was then given 5 ml of a 10% ammonia solution while being agitated. According to (Oluyege et al., 2019), the presence of free anthroquinones is indicated by the creation of a pink, red, or violet color.

Test for saponins

The analysis method used the froth test, in which the extracts were diluted with distilled water to a volume of 20 ml and then agitated in a graduated cylinder for 15 minutes. The presence of saponins is shown by the production of foam.

Test for alkaloids

To perform the Mayer's test, two drops of the potassium mercuric iodide solution were applied to 2 g of the plant sample extract. Alkaloids are present when a white, creamy precipitate appears

Test for steroids

Aqueous H_2SO_4 was added after treating 1ml of the extract with acetic acid. Steroids are present when a coloration turns red

Test for Phenols

To 1 ml of solvent extracts, 2 ml of distilled H_2O was added. To this, a few drops of neutral 10% $FeCl_3$ solution was added. Formation of a dark green colour indicated the presence of phenolics

Test for glycosides

Keller Killiani test was carried out. A solution of 0.5 ml, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 ml of extract. Later, 1 ml of concentrated H_2SO_4 was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycoside

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and potato Dextro Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and potato Dextro Broth (PDB). Each tube containing the broth and the extract was incubated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism been used as a control. All tubes were then incubated 37^oC for 24 hrs. After incubation period, the tube was examined for the

Table 1. Qualitative phytochemical results of leaves extracts of *P. angulata*

S/N	Phytochemicals	Tests	EAE	AE	EE
1	Flavonoids	Extract + $FeCl_3$	+	+	+
2	Phenol	Extract + few drop of 10% $FeCl_3$	+	-	+
3	Terpenoids	Extract+chloroform+conc. H_2SO_4	-	-	-
4	Alkaloids	Mayers/ wagner reagent	+	+	+
5	Steroids	Extract + acetic acid + H_2SO_4	+	+	+
6	Anthraquinone	Extract + 10ml benzene then filter. Filtrate + 5ml of 10% $NH_{3(aq).}$	-	-	-
7	Saponins	Froth / foam test	**+**	**+**	**+**
8	Glycoside	Extract + H_2O+ $NaOH_{(aq)}$	**+**	**+**	**+**
9	Tannins	Extract+ few drops 1% $FeCl_3$	_	**-**	**-**
10	phlabotannins	Extract + 1% HCl in boiling water	_	**-**	**-**

Keys: Absence of phytochemical = (-), Presence of phytochemical (**+**) , **EAE** = Ethyl acetate extract, **AE** = Acetone extract, **EE** = Ethanol extract

Table 2. Qualitative phytochemical results of roots extracts of *P. angulata*

S/N	Phytochemicals	Tests	EAE	AE	EE
1	Flavonoids	Extract + $FeCl_3$	+	+	+
2	Phenol	Extract + few drop of 10% $FeCl_3$	-	+	+
3	Terpenoids	Salkowski Test Extract+chloroform+conc. H_2SO_4	+	+	+
4	Alkaloids	Mayers/ wagner reagent	+	-	+
5	Steroids	Extract + acetic acid + H_2SO_4	+	+	-
6	Anthraquinone	Extract + 10ml benzene then filter. Filtrate +5ml of 10% $NH_{3(aq).}$	+	-	+
7	Saponins	Froth / foam test	**-**	**-**	**+**
8	Glycoside	Extract + H_2O+ $NaOH_{(aq)}$	**+**	**-**	**+**
9	Tannins	Extract+ few drops 1% $FeCl_3$	_	**+**	**+**
10	phlabotannins	Extract + 1% HCl in boiling water	_	**-**	**+**

Keys: Absence of phytochemical = (-), Presence of phytochemical (**+**) , **EAE** = Ethyl acetate extract, **AE** = Acetone extract, **EE** = Ethanol extract

Table 3. Antimicrobial result of the leaves extracts of *P. angulata*

Test organism	EEPA MZI	AEPA MZI	EAEPA MZI	Positive(+) control strept
Bacillus species	12.00	14.00	13.00	24.00
Staphylococcus aureus	11.00	0.00	16.00	23.00
Streptococcus specie	15.00	16.00	24.00	25.00
Plasmodium	21.00	20.00	18.00	26.00

Keys: MZI = mean zone of inhibition, **EEPA =** Ethanol extract *p. angulata,* **AEPA =** Acetone extract *p. angulata* and **EAEPA** = Ethyl acetate extract *p. angulata*

Table 4. Antimicrobial result of the roots extracts of *P. angulata*

Test organism	AEPA MZI	EAEPA MZI	EEPA MZI	+VE control. gentamicin
Bacillus specie	9.00	14.00	9.00	24.00
Staphylococcus aureus	10.00	17.00	11.00	23.00
Streptococcus species	18.00	10.00	23.00	25.00
Plasmodium	18.00	19.00	21.00	26.00

Keys: MZI = mean zone of inhibition, **EEPA =** Ethanol extract *p. angulata,* **AEPA =** Acetone extract *p. angulata* and **EAEPA** = Ethyl acetate extract *p. angulata*

presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without any visible signs of growth was considered to be the minimum inhibitory concentration (MIC) (Rios and Recio, 2005).

Qualitative Phytochemical Analysis of both the Leaves and Roots of *P. Angulata*

The results obtained from the extracts of *Physalis angulata* leaves using ethyl acetate, acetone, and ethanol in the order of increasing polarity. The ethyl acetate, acetone and ethanol extracts of the leaves of *P. angulata* were screened for the presence of some phytochemicals such as alkaloid, anthraguinones, saponnins, stereoids, terpenes, flavonoids, tannins, phenol, glycosides, and phlobatannins. The results obtained shows the absence of phlobatannins, terpenoids, tannins and anthraquinone in all the leaves extracts, this might be due to the non-suitability of those solvents as an extraction solvents for the compounds as reported by Belkacem et al., 2014. Alkaloids, flavonoids, glycocides, steroids and saponins were all present in the leaves extracts. The result show the presence of phenol in both ethyl acetate and ethanol extracts but absence in acetone extract as presented in the 1 above which may be linked to non-suitability and suitability of the solvent for extraction as reported by Belkarem et al., 2014. However In the root extract of this plants, result shows that terpenes and flavonoids are all presence in ethyl acetate, acetone, and ethanol extracts. Hence, alkaloids, anthraquinone and glycosides are all presence in both ethyl acetate and ethanol but absence in acetone extract. Phenol and tannins are presence in acetone and ethanol extract but absence in ethyl acetate extract, phlabotannins and saponins are both absence in ethyl acetate extract and acetone, but are present in ethanol extracts. While steroid is presence in both ethyl acetate extract and acetone extract, but absence in ethanol extracts. The qualitative phytochemical analysis result for the roots is summarized in Table 2 above. These class of secondary metabolites are known to show medicinal activity as well as exhibiting physiological activity, also known to show curative activity against several bacterial due to the presence of some bioactive compounds or secondary metabolites. These metabolites are important mediators of proactive processes causing chronic diseases such as cancer, inflammation, cardio-vascular as well as bacterial and viral diseases as reported by McGraw et al., 2008

Anti-microbial and Anti-malarial activities of *P. angulata*

The results of the antimicrobial and anti- malarial activity obtained from the extracts of both leaves and roots extracts of *Physalis angulata* revealed that both the crude extracts of the leaves and roots inhibited anti-microbial activities against *Bacillus species, Styphylococcus aureus*, *Plasmodium parasites* and *Streptococcus species* except the leaves extract of acetone which does not act against *Styphylococcus aureus* which might be due to the absence of certain phytochemicals. The facts that the plant was active against laboratory isolates are also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms. The anti-malarial and anti-bacterial activities of the medicinal plants can be attributed to the presence of phytochemicals such alkaloids, tannins, terpenes etc because phytochemicals act as disrupting microbial membranes or impairing cellular metabolism which has the ability to cure diseases caused by such bacterial. The presence of this phytochemicals are very helpful for the manufacturing of new drugs that can cure diseases caused by such microorganism. The phytochemical analysis of the *Physalis angulata* is also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases associated with the tested organisms. From the result obtained above using gentamicin as positive control ranging from 23 to 26mm. Ethyl acetate extracts of both the leaves and the roots shows highest activities on streptococcus species, followed by ethanol extract of the roots and ethyl acetate extract of the leaves on plasmodium. The result for the antimicrobial test for both the plant leaves and roots also shows that all the extracts show antimicrobial activities against the clinical isolates either as active, moderately active except acetone extract

which is inactive as presented in Table 3 and 4 above.

CONCLUSION

In summary, the phytochemicals and antimicrobial analysis of *P. angulata* presents new and relevant information regarding the leaves and roots. These has present to us the necessary medicinal information on the site of production and accumulation of plant, potentially one of the most useful compound in this plant. *P. angulata* antioxidant properties confirm the therapeutic potential of this plant. The current findings may support future refinement programs and biotechnological approaches for the optimization of useful compound present in *P. angulata* since good percentage of the leaves and roots extracts of *p. angulata* show anti-microbial activities against the tested organisms.

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AUTHOR'S CONTRIBUTION

Filibus Dogara wrote the manuscript, Dahirul I. Ba'aga and Joseph F. Kpensibe analyzed the data, Abdul S. Sa'ad designed the study. All authors read and approved the final version of the manuscript.

COMPETING INTEREST

The authors declare that there are no competing interests.

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