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Anti-microbial and Phytochemical Characterization of Leaves Extracts of Starburr (*A. hispidum*) collected from Jos North Local Government Area of Plateau State.

***[1]PAM, B; [1]ADAMU, MH; [1]KOLO, AM; [2]MALGWI, DW**

**[1]Department of Chemistry, Abubakar Tafawa Balewa University Bauchi, PMB 0248, Bauchi State, Nigeria*
[2]Department of Chemistry, Modibbo Adama University, Yola, P.M.B 2076 Adamawa State, Nigeria

**Corresponding Author Email: pambulus81@gmail.com*
**ORCID: http://orcid.org/0009-0007-0746-3904*
**Tel: +234814348281*

Co-Authors Email: hmadamu65@gmail.com; amodukolo@gmail.com; danielmalgwi@mau.edu.ng

ABSTRACT: Starburr (*Acanthospermum hispidum*) extract has been reportedly used in North and Western Nigeria and Nigeria at large to treat stomach ache, malaria and abdominal pain. In view of its usage, the objective of this paper was to characterize, carry out the anti-microbial activity and qualitative and quantitative phytochemical analysis of leaves extracts of Starburr of A. hispidum collected from Jos North Local Government Area of Plateau State. Fine powdered sample of A hispidum leaves were extracted using n-Hexane, acetone, ethyl acetate and methanol sequentially in the increasing order of polarity using maceration extraction method. Percentage yield of the leaves of A hispidum. extracts were n-hexane (3.6 %) acetone (4.0 %) ethyl acetate (2.2 %) and methanol (4.10 %). Qualitative phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides and terpenoids. Quantitative phytochemical screening revealed alkaloids (1.456) saponins (3.56) tannins (0.04852) terpenoids(0.05208) for methanol extract alkaloids(1.176) saponins(3.64) tannins(0.0372) terpenoids(0.0398) for ethyl acetate extract and alkaloids(0.328) saponins(3.20) tannins(0.02344) terpenoids(0.01948) for acetone extract. Antimicrobial activity analysis revealed inhibition growth of clinical pathogens which are E. coli, S. aureus, S. typhi, P. aeruginosa, S. pneumonaie, C. albicans as compared with standard of penicillin with inhibition zone of 18 mm and ketokonazol with inhibition zone of 24 mm . The methanol extract with zone of inhibition range of 10 mm to 18.5 mm of the leaves showed a promising potency against the test microbes.

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Since ancient time, man has known instinctively and experimentally how to benefit from the plants and herbs found within a living environment and not only for his food but also in the treatment of his illnesses and diseases (Mintah *et al.*, 2019). Medicinal plants are receiving research attention recently because of their use in ethno medicine treating common disease such as cold, fever and other medicinal claims are now supported with sound scientific evidences. The study on medicinal plants started with extraction procedures that play a critical role to the extraction outcomes (e.g. yield and phytochemicals content) and also to the biological activities and characterization done. A wide range of technologies with different method of extraction is available nowadays. Plants are viewed as chemical factories of nature which constitute natural substances that show or display bioactive properties by producing a distinctive

**Corresponding Author Email: pambulus81@gmail.com*
**ORCID: http://orcid.org/0009-0007-0746-3904*
**Tel: +234814348281*

physiological action on human system when administered (Paul *et al.,* 2021). Natural compounds are evolutionary selected and pre-validated by nature, displaying a unique chemical diversity and a corresponding diversity of biological activities. These features make them highly interesting for studies of chemical, biological, and in the pharmaceutical industry for development of new drugs. Morphine and aspirin are pure natural and semi-synthetic products derived from the opium poppy (Papaver somniferum), and from willow bark, respectively. They were rapidly followed by codeine, digitoxin, quinine and pilocarpine. Traditional medicine, having a long history, is defined as the total sum of knowledge, skills and practices based on the theories, believe and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. The terms complementary alternative/non-conventional medicine are used interchangeably with traditional medicine in some countries (Adiaratou, 2008). Generally, the practices of traditional medicine vary greatly from country to country, and from region to region, as they are influenced by factors such as culture, history, personal attitudes and philosophy. In most cases, their theory and application are quite different from those of conventional medicine. Long historical use and practices of traditional medicine are passed on from generation to generation, including the safety and efficacy. However, scientific research is needed to provide additional evidence of their safety and efficacy. The quality and quantity of the safety and efficacy data on traditional medicine is far from sufficient to meet the criteria needed to support its use worldwide (Adiaratou, 2008). Over 60 % of rural people in Nigeria depend on traditional medicine for the treatment of their ailment (Ayandele and Adebiyi, 2007). In the African region, traditional medicines are used for the management of both sudden, quick, slow and worsen overtime diseases in rural and urban areas. In most cases, the herbal preparations are formulated by the traditional health practitioner upon a visit by a patient or someone taking care of the patient. Plants have been part of man's life since the beginning of time. There are numerous products obtained from plants, most of them not only food, but also crucial to our health need. The use of plants to treat or combat illness is probably as old as mankind (Newman *et al.,* 2012; Kingston, 2011). For centuries native peoples of various cultures have used plants as medicine for all sorts of healings (Hosseinzadeh *et al.,* 2015). Plants were the basis of Indian and Chinese medicine for millennia, and they are still in use up to this day. Nigeria as country has long history for the use of medicinal plant in the treatment of so many diseases conditions (Erhenhi, 2016, Nvau *et al.,* 2011).

Such natural substances obtained from plants are discovered to be less toxic and very effective in terms of fighting diseases. The best-known anti-malaria is from a natural compound (Ginsburg and Deharo, *et al.,* 20l1). Therefore, there is the need to focus on the alternative source of the antibiotics as the pathogenic microbes are gaining resistance against standard antibiotics (Ali *et al.,* 2015). This has led to attempt to discover other antimalarial agents, mainly from plant sources. Medicinal plants may provide may provide anti-malarial drugs directly, as in the case of quinine from *Artemisia annua*. These may supply template molecules on which to base further new structures by organic synthesis.

Phytochemicals are the chemicals that are present naturally in plants. Nowadays these phytochemicals become more popular due to their countless medicinal uses (Banu *et al.,* 20l5). Phytochemicals play a vital role against number of diseases such as arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as "man-friendly medicines".

Isolating these plant substances and elucidating the structures of different chemical constituents obtained from plants is the primary duty in drug discovering process. In some cases, the crude extract is pharmacologically more effective than the purified bioactive compound from the extract (Ginsburg and Deharo, *et al.,* 20l1). The search for plants with broad pharmacological activities but low toxicity has increasingly gained importance in recent years. In Nigeria, different plant species are extensively used in traditional medicine to treat different ailments. One of these plant species is *Acanthospermum hispidum* (Jotham *et al.,* 2019).

Acanthospermum hispidum (Bristly starbur, Goat's head, Hispid starbur, Starbur; (syn. *Acanthospermum humile* Eggers) is an annual plant in the family Asteraceae, which is native to Tropical America. *Acanthospermum hispidum*is considered as an important medicinal plant in Nigeria. The common name of this medicinal plant in Hausa is kashinyawo. *Acanthospermum hispidum*, popularly known as "Espinho-de-cigano" ("Gypsy-Thorn"), has been traditionally used in northeastern Brazil for treating asthma, bronchitis, fevers and as expectorant, as vermifuge and against intestinal pains (Morais *et al.,*

2005; Torres *et al.,* 2005; Agra *et al.,* 2007;2008). Recent public health programs showed the results obtained in treating bronco-asthmatic problems with syrups prepared from this plant have raised awareness about this folk medicine and has provoked a significant increase in demand for this product. It is also possible to find extracts of this plant in stores specializing in natural products and natural medicines, although these preparations often do not meet even the minimal requirements the National Health Service Agency (Anvisa, 2000), in terms of their quality control, proof of efficiency, or safety (Araujo *et al.,* 2002). Even recognizing the normal difficulties involved in working with folk remedies rid products of extensive popular usage, a lack of research and control may expose patients to an inefficient use of such a product or even health risks.

The species is easily identifiable and grows abundantly during the rainy seasons in Nigeria. *Acanthospermum hispidum* is a branched herb up to 60 cm tall. The stems of these plants are covered with bushy hairs and smaller glandular hairs (Habib *et al.,* 2012). These are scattered throughout the stems. Leaves are elliptic, obovate and 1.5 cm to 7 cm long. The *Acanthospermum hispidum* plant bears yellow flowers. Some leaves can be up to 11.5 cm long. The margins of the leaves serrate to sub entire gradually narrowed to base, sessile. The flowers are typical of the Aster or Daisy Family. Each head has 5-9 ray flower. The petals (corollas) of the ray flowers pale yellow and are about 1.5 mm long (Koukouikila-Koussoundaa *et al.,* 2013). The disc flowers in the center of the head are sterile. The fruits are flattened and triangular in shape spiny and 5 cm to l0 cm in length. These fruits are covered with stiff, hooked hairs and have either a straight or curved pair of spines at the top. The bristly appearance and grouping of several fruits in each head provide the most frequently used common name, Bristly starbur. Each fruit, excluding the terminal spines, is 5-6 mm long. The terminal spines are strongly divergent and are about 4 mm long (Vivekanandhan, *et al.,* 2017). Hence, the objective of this paper was to characterize, carry out the anti-microbial activity and qualitative and quantitative phytochemical analysis of leaves extracts of Starburr (A. hispidum) collected from Jos North Local Government Area of Plateau State.

MATERIAL AND METHODS

Solvent used for extraction: The solvent that was used for the extraction are n-Hexane, Acetone, Ethyl acetate and Methanol in that order

Sampling / Authentication of plant; The plant sample (leaves of *Acanthospermum hispidum*) was collected from Jos north native place of Plateau State; the plant material was authenticated by a Taxonomist and Curator at the Federal College of forestry, Jos Plateau State.

Preparation of plant sample; The leaves were dried in a shallow container and then exposing to direct sunlight for two weeks. The well dried plant material was crushed using mortar and pestle. The crushed sample was then stored into a clean polythene bag for further analysis.

C*rude Extraction of Sample;* 200 gram of the powder sample of *Acanthospermum hispidum* was subjected to serial extraction (maceration method at about 30 °C) in n-hexane, acetone, ethyl acetate and absolute methanol for 4 days with constant stirring. The macerated was filtered. The extract was concentrated using rotary evaporator in order to remove the solvent leaving the extract containing small amount of solvent. The extract was then transferred into a pre-weighed beaker and placed in a water bath at about 40 ^{0}C. It will remain in the water bath until a plastic form of the extract is formed (soft extract). All the crude extract obtained were weighed using the weighing balance and the percentage yield of the extract was calculated (Ammar *et al.,* 2021).

Qualitative Phytochemical screening; Chemical test for the screening and identification of bioactive constituent like tannins, alkaloids, terpenoids, flavonoids, cardiac glycosides and saponins of the leaves of A. hispidum was carried out using standard procedure as describe by Harbone (1973); Odebiyi & safowora (1978); Willimson *et al.* (1996); and Benson and Ngbede (2006).

Test for terponoids (Salkowski's test); To 0.5 g each of the extract was added to 2 mL of chloroform. Concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown coloration of the interphase was formed to show the positive result for the presence of terpenoids.

Test for flavonoids (Shinoda test); The extract was mixed with magnesium ribbon fragments and concentrated HCl was added drop wise. Orange, pink or purple coloration indicated the presence of flavonoids.

Test for tannins; In 10 mL of freshly prepared 10 % potassium hydroxide (KOH) in a beaker, 0.5 g of the extract was added and shakes to dissolve. A dirt

precipitate is observed which indicate the presence of tannin.

Test for saponin; About 0.5 g of the plant extracts was shaken with water in test tube, frothing which persist on warming considered as preliminary evidence for the presence of saponin. Add few drops of olive oil to 0.5 g of the extract and vigorously shake, formation of soluble emulsion in the extract indicates the presence of saponin.

Test for alkaloids; Extracts was dissolved individually in dilute hydrochloric and filtered. The filtrate was used to:

i) Mayer's Test: Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a Yellow cream precipitate indicates the presence of alkaloids.

ii) Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in potassium iodide) formation of brown/reddish brown precipitate indicates the presence of alkaloids.

iii) Filtrate was treated with Hager's reagent (saturated picric acid solution): formation of yellow colored precipitate indicates the presence of Alkaloids. Precipitation in any 3 test indicates the presence of alkaloids precipitation in any 3 test indicates the presence of alkaloids (Odebiyi and Safowora 1978; Benso and Ngbede, 2006).

Test for cardiac glycosides (Killani test); About 100 mg of the extract was dissolved in 1mL of glacial acetic acid containing one drop of ferric chloride solution, then under layered with 1mL of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of cardiac glycosides.

Quantitative phytochemical screening: The crude extract of *acanthospermum hispidum* was analyzed quantitatively for total alkaloids, total saponins, total tannins and total terpenoids using standard methods.

Determination of Total Alkaloids: Alkaloids were determined using Harbone Method (Zhang *et al.*,2018).Five grams of the plant sample was weighed and into a 250 mL beaker,200 mL of 10 % acetic acid in ethanol would be added and allowed to stand for four hours. This would be filtered and extract concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until precipitation is complete. The whole solution is then allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue is alkaloid which would be dried and weighed.

Determination of Total Tannins (TTC): Tannin-phenolics was determined by the peri and pompei method (Roghini and Vijayalackshmi., 2018).1 mg/mL was taken in a test tube. The volume was made up to 1 ml with distilled water and 1 mL of water serves as blank. To this 0.5 mL of folins phenol reagent (1:2) followed by 5 mL of 35 % sodium carbonate was added kept at room temperature for five minutes. Blue color intensity read at 640 nm from which the tannin content of the extract would be determined. The total tannin content would be expressed in mg/g of the extract.

Determination of Total Saponins: The extract was grounded and 20 g of the extract was weighed and put into a conical flask and 100 mL of 20 % ethanol was added to the sample (Velavan. 2015). The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55 ^{0} C. The mixture is filtered and the residue was re-extracted with another 200 mL ethyl alcohol. The combined extracts were reduced to 40 mL over a hot water bath at 90 ^{0} C. The concentrate is then transferred into a 250 mL beaker separating funnel 20 mL of diethyl ether is added to the extract and vigorously shaken. The aqueous layer recovered with diethyl ether is discarded and the purification process was repeated. 60 mL of n-butanol was added and the combined n-butanol extracts washed twice with 10 mL of 5 % sodium chloride. The remaining solution was then heated in a water bath and after evaporation. The samples were dried in an oven to a constant weight and the values were expressed in mg/g of extract.

Determination of Total Terpenoids: The dried plant extract 100 mg (W_i) was taken and soaked with 9 mL of ethanol for 24 hours (In du math, *et al.,* 2014). The extract after filtration, was extracted with 10 mL of petroleum ether using separating funnel. The ether extract was separated in pre-glass vials and waited for its complete drying (W_f). Ether was evaporated and the total terpenoid content was measured by the formula

Antibacterial/fungal activity of the extracts: The antibacterial/fungal activity of the leaves of *acanthospermum hispidum* extract for n-Hexane, Acetone, Ethyl acetate and methanol was done using the nutrient agar method with 250 mg of penicillin as control for bacteria and 500 mg of ketokonazol as control for fungi.

Preparation of Culture Media

28 g of the powdered nutrient agar was weighed and 1 litre of deionized water was added to it. It was then allowed to soak for 10 minutes. After that it was sterilized by autoclaving for 15minutes at 121 ^{0}C and allowed to cool to 47 ^{0}C. It was then mixed well and then pouring into plates.

Preparation of the Concentration of the Plant Extracts (mg/ml): 0.4 g, 0.2 g, 0.1 g, 0.05 g for the methanol extract, ethyl acetate extract, acetone extract and n-Hexane extract each was weighed and dissolved in 1mL of DMSO.

Determination of Zone of Inhibition: The determination of the zone of inhibition was done using 250 mg of chloramphenicol as positive control for bacteria and 500 mg of ketokonazol as positive control for fungi. The antibacterial/fungal activities of the crude extract *A. hispidum* (methanol, ethyl acetate, Acetone and n-Hexane extract).

The prepared nutrient agar is poured on the various plate and the pathogenic bacteria's (*E.coli, Salmonella typhi, pseudomonas aeruginosa, streptococcus pneumoniae*) and fungi (*candida albican*).

The pathogenic bacteria and fungi were made in sterile normal saline and adjusted to the 0.5 McFarland's Standard solution. The pathogenic bacteria and fungi poured on the prepared nutrient agar inside the plates and incubated at 37 ^{0}C for 24 hours. The agar well was prepared by using a sterilized cork borer with 6mm diameter. Using a micro pipette the four prepared concentrations 400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml for the methanol extract, ethyl acetate extract, acetone extract and n-Hexane extract was carefully added to the respective wells in the plate media. The antibiotic disc for chloramphenicol and ketokonazol were dispensed with a sterile forceps onto the surface of the prepared nutrient on the plate media. The prepared concentrations of the plant extracts and the antibiotics were allowed to diffuse for about an hour before incubation. The incubation was done in an upright position at 37 ^{0}C for 24 hours. After overnight incubation the zones of inhibitions were measured in mm using caliber and the results were recorded separately. Chloramphenicol (250 mg) serve as control or pathogenic bacteria and ketokonazol (500 mg) serve as control for fungi.

RESULTS AND DISCUSSION

Percentage yield of the extracts: The result obtained from the serial extraction (maceration) of the secondary metabolites of the leaves of *A. hispidum* using n-Hexane, acetone, ethyl acetate and methanol in the order of polarity is shown the table 1.

Table 1: Percentage Yield of Extracts of *A. hispidum*

Extracts	Yield
Methanol	4.1 %
Ethyl acetate	2.2 %
Acetone	4.0 %
n-Hexane	3.6 %

Abdulmalik *et al.* (2020) reported that the percentage yield of the roots of *A. hispidum* using n-hexane, chloroform, methanol in that order yielded 5.6 %, 1.23%, and 8.3 %. Thus from the result above reveals that methanol is good solvent for extraction as the percentage is high. In conclusion the yield of extracts depend on the nature of the phytochemicals present, solvent used, variation in extraction procedures among others (Ammar *et al.*, 2007).

Phytochemical Screening of Extracts from A. hispidum Leaves: Qualitative phytochemical screening: Qualitative phytochemical screening of the components of the plant leaves shows the presence of the phytochemicals tested. The phytochemicals tested and found present includes ; alkaloids, saponins, tannins, glycosides, terpenoids, flavonoids in methanol extract and ethyl acetate extract; alkaloids, saponins, tannins, flavonoids present in acetone; alkaloids, glycosides, tannins, terpenoids present in n-hexane extract. The summary of the result is shown in table 2. Different phytochemicals have found to possess a wide range of activities which may help in protection of chronic diseases. For example, alkaloids protect against bacterial infection (Butler *et al.*, 2004).

Table 2: Phytochemical Screening

	Methanol extract	Acetone extract	Ethylacetate Extract	n-Hexane extract
Alkaloids	+	+	+	+
Saponnins	+	+	+	-
Glycosides	+	-	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	-
Terpenoids	+	+	+	+

Key: + = Present, - = Absent

Saponins protect against hypercholesterolemia and antibiotic properties (Yuliana *et al.*, 2014). Jotham (2019) reported that the phytochemical study methanol extract of the leaves of *A. hispidum* reveals the presence of alkaloids, tannins, saponins, and flavonoids. This research done reveals the presence of alkaloids, tannins, saponins, and flavonoids in methanol extract which is in line with the result obtain by Jotham (2019).

Quantitative phytochemical screening: The quantitative phytochemical screening was performed on alkaloids, tannins, saponins and terpenoids of the leaves of *A. hispidum* leaves extract of methanol, ethyl acetate, and acetone. The summary of the result is shown in table 3.

Table 3: Quantitative Phytochemical Screening

Phytochemical	Methanol extract	Ethyl acetate extract	Acetone extract Constituent (g)
Alkaloids	1.456	1.176	0.328
Tannins	0.048	0.037	0.023
Saponins	3.56	3.640	3.200
Terpenoids	0.052	0.398	0.019

From the results obtained; the maximum alkaloids, tannins, saponins and terpenoids content were found in the methanol extract followed by ethyl acetate and then acetone extract last. S. This result may be due to the nature of the phytochemicals present, solvent used, variation in extraction procedures among others (Ammar *et al.*, 2007). Santhi and Sengottuvel (2006) in their result obtained for the quantitative phytochemical screening of (alkaloids, flavonoids, phenols and carbohydrates) on the leaves, flowers and seeds of *morringa concanensis Nimmo* revealed that the photochemicals were maximum in the leaves compared to the seeds and flowers extract. In this research, the phytochemicals of the leaves quantitatively screened showed that the methanol extract have the highest quantity of the phyto constituents analyzed.

Antimicrobial activity of the crude extracts of *A. Hispidum* leaves: The antimicrobial activities of the *A.Hispidum* leaves extract were tested on *Escherichia coli*, *Salmonella typhi*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albican*. Penicilin (250 mg) as positive control for bacteria and ketokonazol (500 mg) as positive control for fungi. The zones of inhibition are 18 mm for penicillin and 24 mm in diameter for ketokonazol. The summary of the result is shown in table 4.

Table 4: Zones of inhibition (mm)

	Organism/ Conc. mg/ml	*E.coli*	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Staph aureus.*	*Streptococcus pneumonaie*	*Candida albican*
Leaf n-Hexane	400	10.00	8.00	0.00	7.50	0.00	9.00
	200	7.00	6.00	0.00	4.50	0.00	7.00
	100	5.00	5.00	0.00	3.00	0.00	5.00
	50	4.00	3.00	0.00	3.00	0.00	3.00
Leaf Ethyl-Acetate	400	14.50	10.50	0.00	6.50	4.00	10.50
	200	12.00	6.00	0.00	4.50	3.00	8.50
	100	9.00	4.00	0.00	3.00	0.00	5.00
	50	6.00	2.00	0.00	3.00	0.00	3.00
Leaf Methanol	400	18.50	14.00	4.00	10.00	5.00	9.50
	200	15.00	10.00	0.00	6.00	3.00	6.50
	100	10.00	5.00	0.00	4.00	0.00	3.00
	50	7.00	0.00	0.00	3.00	0.00	3.00
Leaf Acetone	400	9.00	6.00	0.00	7.00	3.00	9.00
	200	6.00	5.00	0.00	5.00	2.00	6.00
	100	5.00	4.00	0.00	3.00	0.00	4.00
	50	3.00	3.00	0.00	0.00	0.00	3.00

From the results obtained, the methanol, ethyl acetate, n-hexane and acetone extracts inhibit the growth of E.*coli, S.thyphi, Staph. Aureus* and *Candida albicans* both at high and low concentrations with the methanol extract having the maximum inhibition against *E.coli, S.typhi, Strep. Pneumoniae* and Ethyl acetate extract having the highest inhibition against *Candida albicans*.

The methanol extract showed inhibition on *P.aeruginosa* at highest concentration and no inhibition at other concentrations, while n-hexane,

ethyl acetate and acetone extract showed no inhibition in all concentrations. For *Strep. Pneumoniae*; methanol, ethyl acetate and acetone showed inhibition with methanol extract having the highest inhibition. In view of this all the extracts showed in inhibition against one microbe or the other with the methanol extract showing inhibition against all the test microbes and these may be as a result of the presence of the phytochemicals screened.

Juliet *et al.* (2020) reported on the antimicrobial activities of the leaf extracts of *Zanthoxylum caribaeum L. (Rutaceae)* found that methanol extract have maximum zone of inhibition on the test bacterial and fungi microbes likewise in this research . Abdulmalik *et al.* (2020) reported that among the extracts (methanol, n-hexane and chloroforms of roots of *Acanthospermum hispidum*, all the root extracts were active against the microbes, at least one or more of the pathogenic bacteria and fungi, also in this research all the extracts were found to be active against the test pathogens.

Conclusion: The plant leaves sample was extracted using n-hexane, acetone, ethyl acetate and methanol given the percentage yield in the qualitative and quantitative analysis revealed the presence and quantity of metabolites. Methanol extract from *A. hispidum* leaves possess the highest antimicrobial activity compared to other extracts. Also the presence of these screened secondary metabolites justify the use of the plant in traditional medicine for the prevention of several diseases.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors

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