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ON THE PROCEDURES FOR THE EXTRACTION AND ISOLATION OF FLAVONOIDS PRESENT IN THE METHANOLIC EXTRACT OF LEAVES OF ACANTHOSPERMUM HISPIDIUMDC

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ABSTRACT

This paper presents the procedures of the extraction and isolation of flavonoids present in the methanolic leaf extract of Acanthospermum hispidium DC .The leaves of Acanthospermum hispidium dc was extracted with polar and non-polar solvents. The activecomponents (.i.e. flavonoids) were found in methanol, chloroform, ethyl acetate and n-butanol while methanol, chloroform, ethylacetate and n-butanol contained steroids. From the chromatographic analysis, it was observed that the component 1and 2 have RF values of 0.61 and 0.48. The identification of the components and the specific absorption band were determined by spectroscopic analysis.

Keywords: Acanthospermum hispidium DC, Chromatography, Flavonoids, Methanolic leaf, Phytochemical screening.

1. INTRODUCTION

Since the ancient time, plant medicine is an important part of healthcare system. The heavy reliance on plant medicine is attributed to their relative accessibility, low prices, local availability, and acceptance by local communities and the low number of dispensaries and doctors for healthcare needs especially in rural areas.

Medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in the history of all civilizations [1]. These plants have shown effectiveness in non-insulin dependent diabetes and some have anti-arthritic, anti-rheumatoid anti-flammabtory activity. Medicinal plants and derived products are used for the treatment of major diseases such as typhoid fever, cardiac edema, obesity, high blood pressure, gastritis and migraine [2].

Medicinal plants are biosynthetic laboratory, not only for chemical compounds but for phytochemicals such as glycosides, alkaloids e.t.c which exert physiological and therapeutic effects

[3]. Medicinal plants containing saponins may also help to prevent colon cancer because it can bind cholesterol and thus interfere with cell growth and division.

s. All pages should be numbered. Abbreviations should be defined the first time they are used in the manuscript and a list of abbreviations used should also be provided.

2. ACANTHOSPERMUM HISPIDIUM DC

Acanthospermum hispidium dc belongs to the family of compositae [Asteraceace] that is sunflower family. Its common name is bristly starbur. It also has various vernacular names such as "Ewe onitan meta" in Yoruba and "kassiyawo" in Hausa [4].

Acanthospermum hispidium DC has a broadly club shaped and wavy margined cotyledons and the leaves are oval to triangular, irregularly and coarsely toothed, light green in colour and very broad when it is not mature. But the mature plant is an upright annual with dichotomous [Y-shaped] branching. The Y-shaped form of branching gives the plant one of its common names, slingshot weed. The stems are densely covered with hairs. These hairs can be stiff and bristly or soft and flexible. The leaves have no stalk [sessile] and are opposite each other on the stem.

Bristly starbur are oval to triangular-ovate in shape with a base with a base that narrows rapidly to the stem. Some leaves can be up to 11.5cm long. The margins of the leaves can have irregular teeth or they may be entire and smooth. Like the stems, the upper and lower surfaces and also on the margins. The lower leaf surface is also dotted with glands.

The flowers are typical of the Aster or Daisy family. Each head has 5-9 ray flowers. The petals [corollas] of the ray flowers are pale yellow and are about 1.5mm long. The disc flowers in the center of the head are sterile.

The fruits are flattened and triangular in shape. 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 provides the most frequently used common names, bristly starbur. The terminal spines are strongly divergent and about 4mm long [5].

2.1. History of Bristly Starbur

Bristly starbur appears to have been introduced into Florida in ship ballast at Pensacola in the 1800s. The scientific name of the genus, *Acanthospermum* is from the Greek word acanthi [thorn] and sperms [seed] and refers to the prickly fruit. Hispidumis in Latin means rough, shaggy, prickly or bristly.

2.2. Habitat of Bristly Starbur

This weed is currently a problem in southern Alabama, Southern Georgia, Northern Florida and appears to be spreading southward in Florida. This weed has become naturalized in Africa, India, Australia and West Indies.

Acanthospermum hispidium DC is a dual purpose plant. It has been found to be biologically active against inhibiting the growth of disease-causing micro-organisms such as virus, bacteria, trypanosomanes, yeast and it also has anti-plasmodia activity. In north-eastern Argentina, it is

called fertility regulating plant. It is used for the treatment of stomach upset, wounds and to cure leprosy in Congo [6].

2.3. Phytochemicals

Phytochemicals such as flavonoids, saponins, tannins, glycosides e.t.c are not essential nutrients that are required by human body for sustaining life. There are many phytochemicals and each works differently [7]. Most phytochemicals have anti-oxidant activity and protect cells against oxidative damage and reduce the risk of developing certain types of cancer.e.g. Flavonoids [8].

Phytochemicals such as isoflavones found in soy have hormonal activity.i.e. It imitates human estrogen and helps to reduce menopausal symptoms and osteoporosis [9]. Research suggested that phytochemicals, working together with nutrients found in fruits, vegetables and nuts, may help slow the aging process and reduce the risk of many diseases including cancer, heart diseases, stroke, high blood pressure, cataracts, and osteoporosis and urinary tract infections.

Phytochemicals can have complementary and overlapping mechanisms of action in the body including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system and modulation of hormone metabolism. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy, flavanoids in fruits, allylsulfides in onions, leeks and garlic, cartenoids in fruits and carrots and polyphenols in tea and grapes.

Saponins found in the beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin found in hot peppers, protects DNA from carcinogens and also allicin from garlic has anti-bacterial properties. The leaves of *Acanthospermum hispidium dc* have an anti malaria activity and it is used for the treatment of fever in Brazil [10]

3. EXPERIMENTAL PROCEDURES

These include the materials and methods required in the isolation and characterization of organic compounds

3.1. Materials, Reagents, Glasswares and Apparatus

3.1.1. Materials

- Air-dried and pulverized leaves of *Acanthospermum hispidium DC*
- Glass wool
- Paper tape
- Filter paper

3.1.2. Reagents

- Methanol
- Ethylacetate
- N-hexane
- N-butanol

- Chloroform
- Distilled water
- Conc. Sulphuric acid
- Conc. Hydrochloric acid
- Conc. Sodium hydroxide
- Silica gel
- Acetone

3.1.3. Glasswares And Apparatus

- Glass plates
- Beakers
- Measuring cylinder
- Conical flask
- Test tubes
- Separating funnel
- Round bottom flask
- Condenser
- Glass column
- Distillation apparatus
- Blender
- Retort stand
- Weighing balance
- Stirring rod
- Spatula
- Hot plate
- Oven
- Water bath
- Measuring flask

3.2. Sample Collection

The plant *Acanthospermum hispidium dc* was collected at Idi-Abebe in Ogbomosho, Oyo-State, Nigeria after sunset.

3.3. Preparation of The Sample

The leaves were air-dried after collection for two weeks in the laboratory condition for easy powdering. The dried leaves were ground into fine powder and then weighed.

3.4. Extraction of the Sample

Solvent-solid extraction was carried out on the weighed, air-dried and pulverized leaves of *Acanthospermum hispidium dc.* The weighed sample was soaked with methanol for two days and the

solvent was changed every twenty-four hours, until no extraction was observed. The separation of the residue from filtrate was done by using filter paper. It was followed by the concentration of the filtrate by using distillation method. The concentrated extract was weighed by using weighing balance.

Solvent-solvent extraction was also carried out. The weighed concentrated extract was suspended in the distilled water then extracted with n-hexane, chloroform,ethylacetate and n-butanol sequentially. The mixtures were shaken vigorously and were made to stand for some time for proper separation.

All the fractions gotten the extracts (.i.e. n-hexane, chloroform, ethyl acetate and n-butanol fractions) are labelled properly and concentrated by distilling off the solvent using water bath.

3.5. Chromatography

We use two types of chromatography methods to separate the constituents that were present in the leaves extract .i.e. column chromatography and thin-layer chromatography.

3.5.1. Thin-Layer Chromatography (TIC)

TLC was used to ascertain the number of constituents present in the extract and to determine their purity. TLC was also used to determine the solvent mixture that will affect the separation of the components.

- **Preparation of Silica Gel Plates**

50g of silica gel powder was weighed into a conical flask, 100ml of distilled water was added and the resulting solution was shaken vigorously in order to avoid lumps.

The white smooth paste mixture was spread over the glass plate and was allowed to solidify. The coated glass plates were put inside oven for 1-2 hours at 110^0C to ensure further solidification.

- **Spotting of the Plates**

This is done with aid of capillary tubes to introduce few drops of the dissolved sample extract unto the coated plate, allowing each drop to dry before adding another drop.

- **Developing of the Plates**

After the solvent had travelled some distance across the plate, the plate was removed and allowed to dry and then viewed in Iodine tank.

The separated components appeared as dark yellow spots in the Iodine vapour. The retention values were calculated by making use of the distance moved by the solvent and the distance moved by the component.

$$RF = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}}$$

3.5.2. Column Chromatography

This was done to isolate and purify the constituents present in the extracts.

3.5.2.1. Packing of Column

- Dried glass column was held in place by retort stand and was sealed with glass-wool.
- The column was packed with n-hexane and silica gel as adsorbent and the column was tapped in order to avoid air-bubbles.
- 5ml of the extract was introduced into column then solvent mixture (eluent) in proper ratio was added into the column.

Several fractions were obtained, concentrated and their purity was determined by using thin-layer chromatography. The impure fractions were further re-chromatography using a different solvent mixture.

3.6. Phytochemical Screening

The following tests were carried out on crude extracts and solvent-solvent extracts (.i.e. ethylacetate, n-butanol, chloroform and distilled water extracts) of *Acanthospermum hispidium dc* in order to ascertain the presence of these phytochemicals: flavonoids, tannins,glycosides, saponins, steroid and alkaloid.

- **Test for Flavonoids**

1cm^3 of 10% NaOH was added to 3cm^3 of the extract. A yellow colouration indicates the presence of flavonoid.

- **Test for Tannins**

1cm^3 of freshly prepared 10%KOH was added to the extract, a dirty white precipitate indicates the presence of tannins.

- **Test for Glycosides**

To 1cm^3 of the extract in the test tube, 10cm^3 of 50%H_2SO_4 was added. The mixture was heated in boiling water for 15minutes. 10cm^3 of fehling's solution was added, a brick red precipitate indicates the presence of glycosides.

- **Test for Saponins**

Emulsion test: 5 drops of olive oil was added to 3cm^3 of the extract in a test tube, the mixture was vigorously shaken. A stable emulsion indicates the presence of saponins.

- **Test for Steroid**

Salkowski test; 5 drops of concentrated H_2SO_4 was added to 1cm^3 of the extract. Red colouration indicates the presence of steroids in the extract.

- **Test for Alkaloids**

To 1cm^3 of the extract, 2 drops of Maeyer's reagent was added. A creamy precipitate indicates the presence of alkaloids in the extract.

3.7. Spectroscopic Analysis

Infra-red (IR) analysis was carried out on the isolated compounds in order to determine their structure while Ultra-Violet (UV) was used to determine specific absorption band that were characteristic of isolated compounds.

4. RESULTS AND DISCUSSIONS

4.1. Results of The Crude Extract Obtained From The Leaves of *Acanthospermum Hispidium Dc* [1]

Weight of the powdered sample = 1000g

Weight of the dried beaker = 174.5g

Weight of beaker + sample extract = 272.8g

Weight of the extract sample = 98.3g

$$\%\text{Yield of extract} = \frac{\text{weight of extract}}{\text{Weight of powdered sample}} \times 100$$

$$= \frac{98.3}{1000} \times 100$$

$$= 9.83\%$$

4.2. Result from Chromatographic and Spetroscopic Analysis

Retention factor which is the distance moved through the stationary phase to that of mobile phase. Column chromatography gave different fractions and these fractions were concentrated and their purity was determined by using thin- layer chromatography. The fractions obtained and their R_F values as follows:

Table-1. R_F values and weight % for isolated compounds

Fractions	R_f Values	%Weight
Component 1	0.61	0.08
Component 2	0.48	0.15

Table-2. IR data of compound 1

Frequency	Probable Assignment
2988.30	C-C stretch
1772.53	5 membered ring lactones
1646.35	C=O stretch
1042.64	C-O stretch
1436.38	C-CH_3 bend vibration

Table-3. IR data of compound 2

Frequency	Probable Assignment
3450.21	Phenolic OH stretch
2936.45	C-H stretching vibration
1772.19	5 membered ring lactone
1636.91	C=C stretch
1042.54	C-O stetch

Table-4. UV data of compound 1

Wavelength	Absorbance
211	0.115
364	0.105
250	0.101
301	0.100

Table- 5. UV data of compound 2

Wavelength	Absorbance
193	0.615
199	0.443
394	0.215
361	0.214

4.3. Results Of Phytochemical Screenings Barthel and Reuter [3]

Table-6. Phytochemical screening of the crude extract

Extracts	S	F	S	T	A	G
Methanol	+	+	-	-	-	-
N-hexane	-	-	-	-	-	-
Chloroform	+	+	-	-	-	-
Ethylacetate	+	+	-	-	-	-
N-Butanol	+	+	-	-	-	-
Water	-	-	-	-	-	-

Sr- Steroids, F- Flavonoid, S- Saponins, T- Tannins, A Alkaloid, G- Glycoside

Table-7. Percentage Weight Of Fraction With Their Colours.

Fractions	%Weight	Colour
N-Hexane	50.67	Black
Chloroform	2.31	Green
Ethylacetate	5.27	Yellow
N-Butanol	4.62	Gold
Water	37.13	Brick red
	100.00	

Table-8. Phytochemical test on fractionated extracts

Test	Observation	Inferences
N-hexane extract + 10%NaOH	No yellow colouration	Flavonones absent.
N-hexane extract + conc. H_2SO_4	No orange colouration.	Steroid absent.
Chloroform extract + 10%NaOH	Yellow colouration	Flavanones present.
Chloroform extract + conc. H_2S0_4	Orange colouration	Steroid present.
Ethyl acetate extract + 10%NaOH	Yellow to orange	Isoflavones present.
Ethylacetate extract + conc.H_2SO_4	Yellow to orange	Steroid present.
N-butanol extract + 10%NaOH	yellow to orange	Flavones present.
N-butanol extract + conc. H_2SO_4	orange to crimson	Steroid present.
Water extract + 10% NaOH	Yellow to orange	flavonols and flavones
Water extract + conc. H_2SO_4	Yellow to orange	present flavanols and flavones present..

Table-9. Test for class of flavonoids

Test	Observation	Inferences
Chloroform extract + aqueous NaOH	Yellow	Present
N-butanol extract + aqueous NaOH	Yellow	Absent
Ethylacetate extract + Aqueous NaOH	Yellow to orange	Present

5. CONCLUSION

A kilogramme of powdered sample of *Acanthospermum hispidium dc* was soaked with methanol for two days, 98.3g extract was obtained. The presence of steroid and flavonoid were discovered in methanol, trichloromethane, ethylacetate and n-butanol extracts while saponins, tannins, alkaloid and glycosides were absence in the extracts. Flavanoid was also discovered in water extracts and no phytochemical was found in n-hexane.

Phytochemicals screening was carried out on methanol, n-hexane, trichloromethane, ethylacetate, n-butanol and water extracts.

The isolation and purification of the components were done by using column chromatography and thin-layer chromatography while infra-red analysis was carried out for the

identification of the components and the specific absorption band was determined by ultra-violet analysis. Component 1 and component 2 are flavonoids but because of incomplete spectroscopic data, the structures cannot be determined [10].

REFERENCES

[1] F. V. Dfeudis, "Pharmacological activities and clinical applications of Ginkgo bloba extract," *Elsevier Editions Scientifiques*, 1991.

[2] J. Kleijinen and P. Kniposchild, "Ginkgo biloba lancel," pp. 1136-1139, 1968.

[3] A. Barthel and G. Reuter, "PA," vol. 23, p. 26, 1968.

[4] W. H. David and V. V. Vernon, "Article on bristly starbur," *Acanthospermum Hispidium DC*, pp. 236-243, 1997.

[5] E. Cartagena, B. Barton and A. Cataiancai, "Germacranolides and a new type of guaiogolide from acanthospermum hispidium dc," *INAT Prod*., vol. 6310, pp. 1323-1328, 2000.

[6] A. K. Summerfield, T. C. Metteileiter and H. J. Rgaihas, "Antiviral activity of a extract from leaves of tropical plant, acanthospermum hispidium dc," *Antiviral RES*., vol. 361, pp. 55-62, 1997.

[7] K. Nakanishi, *Natural products chemistry*, New York: Academic, 1974.

[8] H. Harborne, *Comparative biochemistry of the flavonoids*: Academic press, 1967.

[9] P. R. Cheeke, "Suprising benefits of desert plant," pp. 57- 64, 1998.

[10] R. T. Abe, "Extraction and isolation of flavonoids present in the methanolic extract of leaves of acanthospermum hispidium dc," *Global Journal of Medicinal Plant Research*, vol. 1, pp. 111-123, 2013.