Sudan University of Science and Technology

College of Science

Department Scientific Laboratories

Extraction and Analysis of Hydnora abyssinica Root (Tartose)

Graduation project submitted for fulfillment of the requirements of B.Sc (honors) in scientific labs (chemistry)

By

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استهلال

بسم الله الرحمن الرحيم

قال تعالى (سنريهم اياتنا في الافاق وفي انفسهم حتى يتبين لهم انه الحق أولم يكف بربك انه على كل شئ شهيد) صدق الله العظيم

فصلت آية (53)

DEDICATION

To our lovely parents

To our brothers and sisters

To all our friends

Thank you for your support, help and encouragement in pursuing our dreams.

ACKNOWLEDGEMENTS

First of all our thanks would go to Allah for giving us strength and help to complete this work

We would like to thank Dr. Omar Adam Gibla for his supervision and advices during the performance of this work.

Our thanks would extend to our families and all those who give us help and encouragement throughout our work duration.

Special thanks to chemistry lab members in ministry of minerals, University of Khartoum central lab, Ministry of industry centre for research and the national research centre for their unlimited support.

Abstract

The aim of this study was to determine the composition of hydnora abyssinica root extract sample, from chemical and antibacterial site of view.

The root sample was extracted by absolute methanol (99.8%).

X-Ray fluorescence technique was used for determination of some minerals. The analysis showed the presence of calcium Ca, magnesium Mg, sodium Na, potassium k, aluminum Al, phosphors P, zinc Zn, nickel Ni, copper Cu ,iron Fe , sulphur S, rubidium Rb , silicon Si, chloride and bromide.

Infrared spectroscopy technique was used for main functional group occurrence. This showed the presence of O-H, C-O, C=C, C-H, benzene ring).

The agar well diffusion method was used to determine the susceptibility of bacterial strains to the extract of the plant. The antimicrobial activity of Hydnora abyssinica against some types of bacteria (Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa Bacillus subtilis) demonstrated a mean zonediameter of inhibition ranging from 12 to 22 mm and that mean, the extract is active against bacteria.

Ash content was determined and it was found to be 3.81%

المستخلص

الهدف من هذه الدراسة هو تحديد مكونات جذور الهيدنورا ابيسينيكا المعروف محليا بالترتوس استخدم الميثانول المطلق (99.8 %) كمذيب لفصل المكونات واجريت الاختبارات على المستخلص من وجهة النظر الكيميائية والباكترولوجية.

استخدمت تقنية فلورة الاشعة السينية لمعرفة المكونات المعدنية وقد اظهر ذلك وجود كل من الكالسيوم Ca ، المغنيزيوم Mg ، الصوديوم Na ، البوتاسيوم K ، الالمونيوم Al ، الفسفور p ، الزنك Zn، النيكل Ni ، النحاس Cu ،الحديد Fe ، الكبريت S ، الربيديوم Rb، السيليكون Si الكلور Cl , البروم Br.

اختبارات تحليل مطيافية الاشعة تحت الحمراء اظهرت وجود الزمر الوظيفية (O-H , C-H , C=C , C-O benzene ring)

الاختبارات الباكترولوجية اظهرت فعالية المستخلص ضد بعض انواع البكتريا وهي (Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa Bacillus subtilis)

وحددت كذلك نسبة محتوى الرماد والتي وجد انها تساوي3.81%.

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1. INTRODUCTION

1.1 Historical background

Hydnora abyssinica A. Br. (family H/ydnoraceace) is the accepted name in African Plant Checklist and Database (Hala, 2010).

Hydnora Abyssinica is a parasitic plant. It has such an unusual physical appearance that one would never say it is a plant. It looks astonishingly similar to fungi and is only distinguishable from fungi when the flower has opened. (Williamson, G. 2000).

The plant body is completely leafless, void of chlorophyll and is brown-grey. As it ages, the plant turns dark grey to black. A network of thick rhizophores or subterranean stems and roots traverse the soil around the host plant. These fleshy, angular, warty stems bear a series of vermiform (shaped like a worm) outgrowths commonly referred to as roots, ± 10 mm that connect to the roots of the host. The plant body is only visible when the developing flowers push through the ground. (Williamson, G. 2000).

The buds of these bisexual flowers develop underground and eventually emerge, reaching a height of about 100 to 150 mm. The flower is spherical, brown on the outside and bright salmon to orange on the inner surface, and has 3 or 4 thick, fleshy, perianth lobes, fused in the beginning but later rupturing vertically as the flower matures, at which point the bait bodies are exposed. The exposed inner surface of the perianth lobes are covered by many stout bristles. The stamens are peculiar and situated halfway down the perianth tube which is 10-20 mm wide. Flowers may not appear for a number of years until sufficient rain

has fallen. The fruit is a subglobose (half-round), underground berry with a thick, leathery wall epidermis/skin. The seeds are numerous and small and embedded in a gelatinous, edible fleshy pulp which is rich in starch. (Williamson, G. 2000).

1.1.1 Conservation Status

Hydnora Abyssinica is not endangered and although not often encountered, is thought to be fairly common in semi-arid vegetation.**(** Williamson, G. 2000)

Fig 1.1.a **(**Un- opened flower)

Fig 1.1.b (Un- opened flower broken by animal) Tarangire, Tanzania

Fig (1.1): Hydnora abyssinica

1.1.2 Habitat

In acacia woodland and scrub, Acacia-Commiphora scrub and grassland with scattered Acacia spp, usually on black cotton, clay and sandy alluvial soils, also on rocky slopes Parasitic on Acacia spp. But also reported on Albizia, Delonix and probably Commiphora spp.(Hala, 2015).

1.1.3 Derivation of name and historical aspects

There are two genera that belong to the Hydnoracea family; Prosopanche which is restricted to the eastern half of South America and Hydnora which is a strictly African genus. Collectively Prosopanche and Hydnora contain between 10 and 15 species. (Williamson, G. 2000).

The genus name Hydnora is taken from the Greek word, hydnon, which means fungus-like and refers to the resemblance that this species has to the fungus genus Hydnum. The specific name Abyssinica means from Africa. (Williamson, G. 2000).

For hundreds of years, jackal food was well known by the indigenous inhabitants of southern Africa. It was only from 1774 that the plant was introduced to science in the western world by the father of South African botany, Carl Thunberg. This very active and well-travelled Swedish botanist discovered and collected the first known records of H. Abyssinica near Calvinia in the Hantam District of South Africa. When he first saw the plant he thought it was a fungus and named it Hydnus after the fungus group. Subsequently the genus name changed to Hydnora. (Williamson, G. 2000).

1.1.4 Ecology

No leaves or chlorophyll are visible on Hydnora Abyssinica plants. Plants only become visible when the flowers protrude through the soil after good rains have fallen. Under favorable conditions it takes at least one year for a bud to develop into a mature flower. The flowers bears no resemblance to normal flowers except for its bright salmon to orange red color on the inside which fulfils the same purpose as normal flowers which is to advertise the plant. White bait bodies are found on the inner base of the flowers. The bait bodies play a very important role in the life cycle of the plant. They omit a putrid odour to attract various carrion beetles and other insects which become trapped in the flowers. Numerous stiff bristles are found on the inner surface of the perianth lobes which restrain the trapped insect from escaping. After feeding on the bait bodies, the trapped insect drops down the flower tube onto the anthers collecting pollen all over its body. It then drops even further down onto the soft cushion-shaped stigma thus pollinating the flower. (Williamson, G. 2000).

Fully grown, ripe fruits of Hydnora Abyssinica may measure up to 80 mm across and contain up to 20 000 seeds per fruit. The brown pinhead-sized seeds are embedded in an edible gelatinous pulp with a slightly sweet and starchy taste. These fruits are favored by mammals such as porcupines, moles, baboons, jackal and also birds. The seeds are not digested and

thus are in an ideal state for germination when excreted by animals. . (Williamson, G. 2000).

Not much is known about the germination of the seeds except that the seeds are more likely to germinate in close proximity to the host plant. The germinated seed develops a primary root (primary haustorium) which establishes the first attachment point to the host. After the plant has grown and spread, it may develop several secondary haustoria, attaching itself to the same or different host nearby. . (Williamson, G. 2000).

The plants have modified their roots in such a way as to ensure invasion of the potential host's tissue. It is thought that the plant excretes powerful enzymes that dissolve away the hard tissue of the host in order to attach itself. The point of attachment is called the haustorium (haustorial roots) and is similar to a graft where two separate members of the union develop together in harmony. Once established, the plant is able to live off the nourishment from the host and quickly develops a matrix of underground stems from which the flower buds develop and eventually emerge above the ground. (Williamson, G. 2000).

1.1.5 Uses and cultural aspects

The fruit of Hydnora Abyssinica is said to be a traditional Khoi food, but there are no recorded details to confirm this. The fruit is delicious when baked on a fire and has a sweetish taste. Jackal food is used in a series of Cape dishes as recorded in the recipe book of Betsie Rood, Kos uit die veldkombuis (Rood 1994). One of the recipes describes how the fruit pulp can be mixed with cream to make a delicious dessert. The fruit is extremely astringent and has been used for tanning and preserving fishing nets. Diarrhea, dysentery, kidney and

bladder complaints are all treated with infusions and decoctions of Hydnora Abyssinica. Infusions used as a face wash also treat acne. (Williamson, G. 2000).

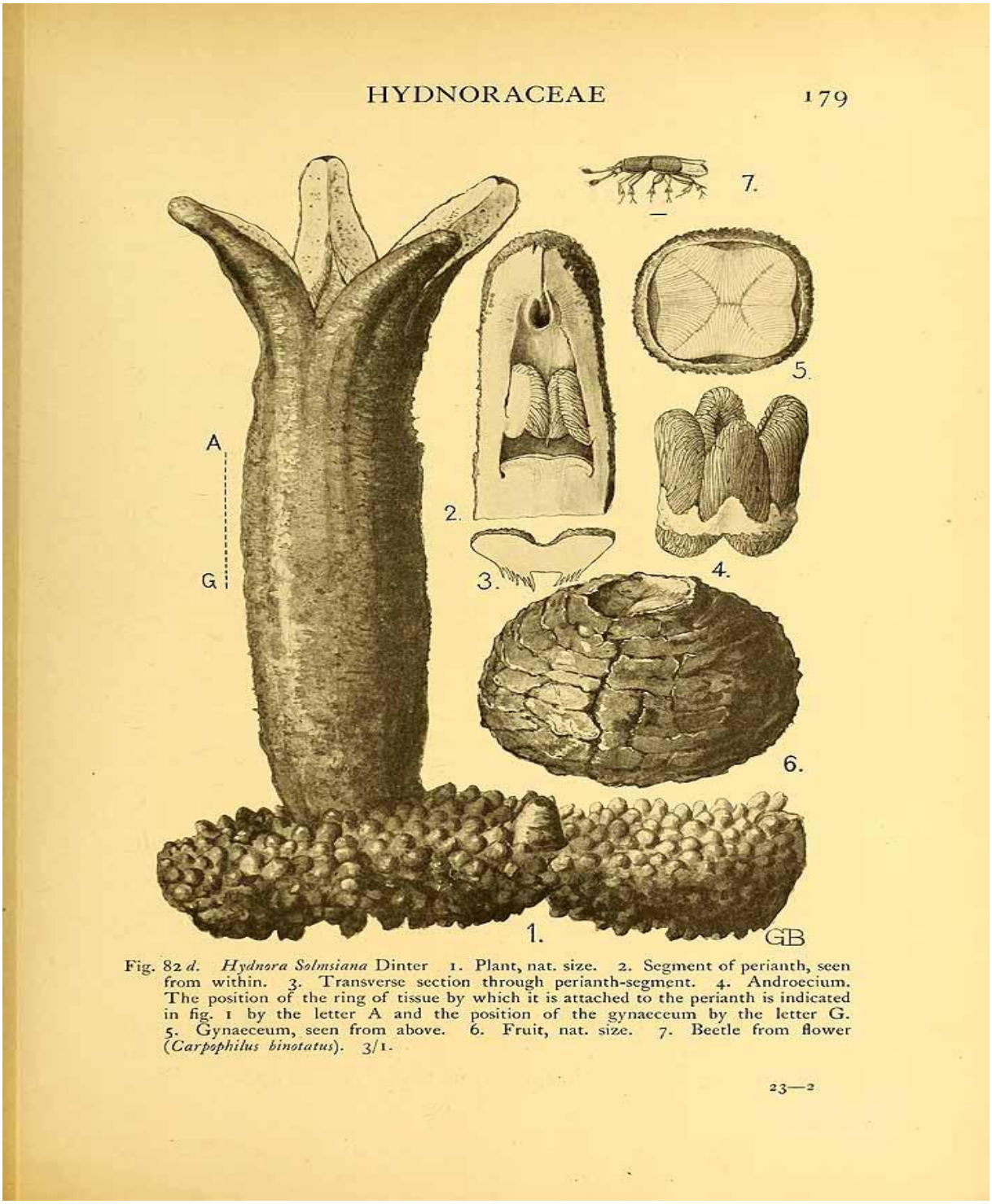


Fig 1.2: hydnora parts

1.1.6 Distribution

The plant is known in Kenya, Tanzania, Uganda, Botswana, Zimbabwe, Ethiopia, Somalia, South Africa, Sudan, Swaziland, Tanzania, Uganda, Yemen, the Democratic Republic of Congo, Namibia, Namibia, Rwanda and Eritrea. (Hala, 2010).

1.1.7 Scientific classification

Kingdom: Plantea

Clade: Angiosperms

Order: Piperales

Family: Hydnoraceae

Genus: Hydnora

Species: Hydnora abyssinica

1.1.8 Types of hydnora

(L. J. Musselman, ODU).

- Hydnora Africana**.**
- Hydnora johannis.
- Hydnora solmsiana.
- Hydnora esculenta**.**
- Hydnora triceps.
- Hydnora abyssinica**.**

Fig 1.3 showed some types of hydnora.

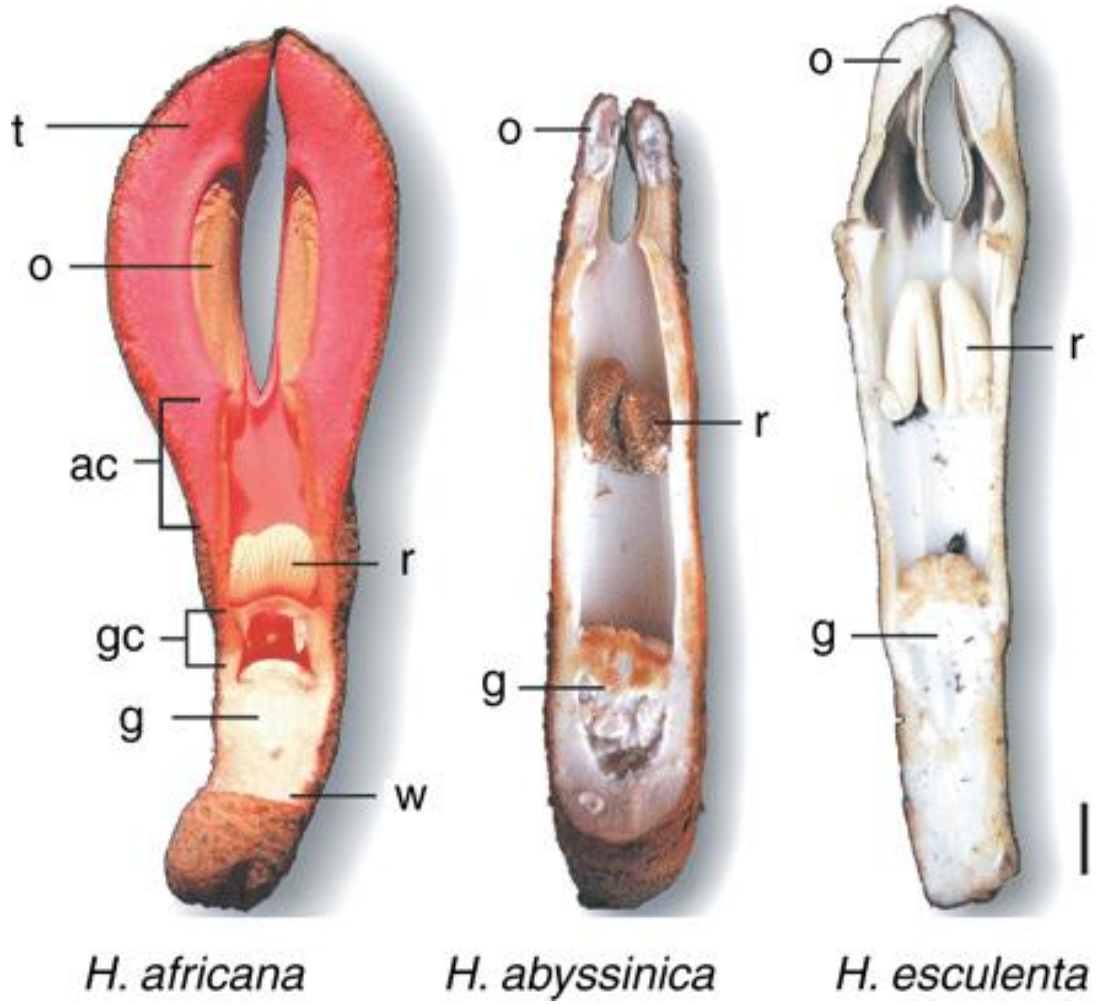


Fig 1.3

Flowers of *Hydnora africana*, *H. abyssinica* and *H. esculenta* largely share the same basic plan. However, the osmophores (o) of *H. africana* are recessed on the interior surface of each tepal (t) but are apical in *H. abyssinica* and *H. esculenta*. The *Hydnora* chamber flower is comprised of androecial (ac) and gynoecial (gc) chambers divided by an antheral ring (r). The stigma is positioned on the floor of the gynoecial chamber and subtended by the gynoecial tissue (g). The floral wall (w) surrounds the gynoecial chamber and gynoecial tissue, and includes the base of the flower. Scale bar = 2 cm.

1.2 Previous studies

1.2.1 Tanning material of hydnora Africana thunb

The study was made on the water solution of this plant. The water solution was found to contain compounds

in the Free State. These were identified as sucrose, glucose, catechol, pyrogallol and phenol. The water solution was found to contain hydrolysable and condensed tannins in the ratio 3:1 by weight. The structure of the hydrolysable tannin was elucidated and was found to contain one glucose molecule to twenty Gallic acid molecule. In other words it was found to be pentagalloyl glucose. (Gamal E.B EL Ghazali, Wail E. Abdalla, 2000).

1.2.2 The antibacterial, phytochemicals and antioxidants evaluation

The study by used the root extracts of Hydnora Africana Thunb. Used as anti-dysenteric in Eastern Cape Province, South Africa.

To determine the anti-dysenteric, phytochemicals and anti-oxidative properties of the root extracts of Hydnora Africana. The use of plants for the treatment of dysentery and other diseases in traditional medicine has increased on the basis of these rich traditional medicine systems. Series of pharmacological tests are recommended since the aetiology of many diseases may be due to more than one factor.

Methods: The agar well diffusion method was used to determine the susceptibility of bacterial strains to crude extracts of the plant. The minimum inhibitory concentration was determined by the micro dilution test. The presence of phytochemicals and antioxidant was also assessed using standard methods.

Results: The antimicrobial activity of H. africana against all the tested organisms demonstrated a mean zone diameter of inhibition ranging from 0 to 25 mm. The MIC of the extracts ranged from 0.071 to 5.0 mg/mL. Antioxidant activity showed lower ferric reducing activities, moderate nitric oxide, moderate DPPH and higher ABTS scavenging activities of the plant. Phytochemical assay revealed the presence and equivalent quantity of alkaloids, tannins, flavonoids, saponins and phenolic acid in the extracts. The water and methanol extracts were also shown as the best solvents of extraction for the phytochemicals. (OA Wintola and AJ Afolayan, 2105) .

1.2.3 Immunosuppressive phenolic compounds from Hydnora abyssinica A. Braun

The study of the whole plant ethanolic extract and its isolated compounds.

Methods: Lymphocyte proliferation, chemiluminescence and superoxide reduction assays were used for immunomodulatory evaluation. While, MTT (3–(4, 5-dimethylthazol-2-yl)-2, 5-diphenyl tetrazonium bromide) test was performed on 3 T3 cell

line clone in order to evaluate the cytoxicity effect of the extracts and isolated compounds of phenolic derivatives which were carried out by chromatographic techniques.

Results: Catechin, (1), tyrosol (2) and benzoic acid, 3, 4, dihydroxy-, ethyl ester (3) compounds were isolated from HA ethanolic extract which revealed potent immunosuppressive activity against reactive oxygen species from both polymorph nuclear cells (PMNs) (45–90 % inhibition) and mononuclear cells (MNCs) (30 -65 % inhibition), T lymphocyte proliferation assay (70–93 % inhibition) as well as potent inhibitory effect against superoxide production (42–71 % inhibition) at concentrations of 6.25–100 μg/mL. Catechin (1) was found the most potent immunosuppressive agent among all constituents examined. (Waleed S. Koko, Mohamed A. Mesaik, Rosa Ranjitt, Mohamed Galal and Muhammad I. Choudhary, 2015).

1.2.4 Anti-diarrhoeal Activity of Hydnora abyssinica

This study was designed to investigate the anti-diarrhoeal activity of the orally administrated extract of Hydnora abyssinica roots at different doses to rats. The anti-diarrhoeal potential of plant extract was tested in rats by faecal mass inhibition. The toxicity of the plant extract was also tested at different doses to rats via oral route.

The aqueous extract of *Hydnora abyssinica* was tested using twenty- four Albino rats arranged in five groups (control+ 3 test groups + loperamide group) receiving oral dose rates of 100,

200 and 400 mg extract/kg body weight/rat, 3 mg loperamide (reference anti-diarrhoeal drug)/kg body weight/rat and 1 ml/kg body weight/rat of normal saline (control). All individuals were given orally, 30 minutes subsequent to extract administration, acute diarrhoea inducer (1 ml castor oil /kg body weight/rat). Cumulative wet faecal mass was weighed for assessment of diarrhoea state at 4 and 6 hours.

The safety of the extract was also tested, using twenty Albino rats distributed in four groups (control + 3 test groups) given oral dose of 400,

800 and 1600 mg extract/kg body weight/rat/day, continued for seven days.

The aqueous extract of *Hydnora abyssinica* showed significant ($P<0.05$) anti-diarrhoeal activity against castor oil induced diarrhoea in all rats with reduction rates of 46.78%, 63.21% and 74.68% for the doses 100, 200 and 400 mg/kg body weight respectively at 4th hrs post treatment and these doses rates recorded inhibition rates of 27.42%, 50.88% and 60.13% at 6th hours respectively post treatment.

Clinical signs were observed regularly. Sera were analyzed for enzymatic activities of AST, ALT and metabolic indicators such as total protein, albumin, sodium, potassium and calcium. Also, changes in values xi of RBCS, Hb, PCV, MCV and MCHC were recorded. The weight of faeces was recorded too for assessment of diarrhoeal inhibition.

In both experiments, clinically no signs of toxicity were observed, no deaths or gross changes in the examined vital organs.

Metabolic indicators; total protein, potassium and calcium demonstrated significant increase while sodium and albumin showed no significant changes at dose 400 mg/kg.

In haematological parameters there were significant decrease in values of RBCs, PCV and MCHC while Hb concentration showed significant increase. MCV values indicated no changes.

Plant extract's safety showed no significant change in the serum (ALT) activity while in (AST) activity there was significant increase. In serum metabolites, there were no changes in the concentration of total protein and albumin when compared with the un-dosed control group at the end of the experiment. The concentration of sodium and potassium showed significant increase.

There were significant decrease in the concentration of haematological parameters tested; RBCS, Hb and PCV. MCHC showed significant increase, while MCV values demonstrated no changes. (Hala, 2010).

1.2.4 Structural Characterization and Physical Properties of Hydnora Africana

The physical properties of several parts of Hydnora triceps (Cover, root and seeds) were investigated. The structure of four

parts of Hydnora was investigated using X-ray fluorescence (XRF) and X-ray diffraction (XRD). The XRD indicated that structures of all types are amorphous. The XRF results for the cover and seeds indicated that, both are contained Potassium (K), Iron (Fe), Copper (Cu), Zinc (Zn), Lead (Pb), Barium (Br), Strontium (Sr), Yttrium (Y). Optical properties of Hydnora were carried out using Fourier Transformation infrared spectroscopy (FTIR) and Ultra violet spectroscopy (UV). The FTIR spectra showed a broad and strong absorption band in the range 1100-2920 cm–1, and these absorptions were assigned to the different stretching vibrations. The absorption for Hydnora cover, root, seed 1 and seed 2 was found to be 1.8, 3.0, 0.83 and 0.37 a. u, while the wave length was found to be 235, 220, 200 and 195 nm, respectively. The energy band gap is calculated and found to be 5.46, 4.96, 5.50 and 5.60 EV, for cover, root, seeds 1 and seeds 2 of Hydnora, respectively. (Mohamed A. Siddig, 2015).

2. Materials and Methods

2.1 Plant material (collection of plant)

The selected plant species was collected from its natural habitat in the central Sudan (wad madani) around Acacia.

2.2 chemicals

- Methanol

(99.8%, 0.79 g/cm³, ALPHA CHEMIKA median INDIA).

2.3 Instruments

- Furnace.

-Mechanical Shaker.

-XRF- Spectrometer Cd_109 XRF spectrometer system.

-Rotary evaporator.

-FTIR – 8400S, Shimadzu.

2.4. Methods of analysis

2.4.1 Determination of ash content

A crucible was heated at 600c, cooled in disicator and weighted (w1). 5 grams of plant material was weighted in the crucible (w2). And ignited at 600c in the farnce until free from carbon, cooled in disicator and weighted (w3). Then the total ash % was calculated as follows:

$$\text{Total ash \%} = 100*(w3- w1)/ (w2- w1)$$

2.4.2 Extraction method

40.047 grams of whole plant material was air-dried under the shed, grounded, and extracted by methanol on a shaker for 48 h with. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. The extracts were further concentrated to dryness under reduced pressure at 37 °C using a rotary evaporator to remove

the solvents (OA Wintola and AJ Afolayan, 2015). The extract obtained was powder and weighed (19.2968gm), the yield percentage was calculated. Then the extract obtained was stored in a closed container till use.

The yield % = (w2/w1)*100

2.4.3 Antibacterial test

Bacterial microorganisms:

Bacillus subtilis	NCTC 8236 (Gram + ve bacteria)
Staphylococcus aureus	ATCC 25923(Gram +ve Bacteria)
Escherichia coli	ATCC 25922(Gram -ve bacteria**)**
Pseudomonas aeruginosa	ATCC 27853 (Gram -ve bacteria)

National Collection of Type Culture (NCTC), Colindale, England.
American Type Culture Collection (ATCC) Rockville, Maryland, USA.

2.4.3.1 Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number

of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.4.3.2 Testing of antibacterial susceptibility (Disc diffusion method)

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2.4.4 XRF analysis

The XRF was performed using Cd-109 XRF spectrometer system

2.4.5 FTIR analysis

The powder of sample was mixed with a little amount from potassium bromide (KBr).this powder mixture then pressed in mechanical press to form translucent pellet through which the beam of spectrometer can pass.

3. Results and Discussion

3.1 Results

3.1.1 Ash content

Table (3.1) Ash content

W1	W2	W3	%
48.5061	53.5584	48.6986	3.81

3.1.2 Extraction percentage

The yield % =48.19%

3.1.3 Antibacterial activity

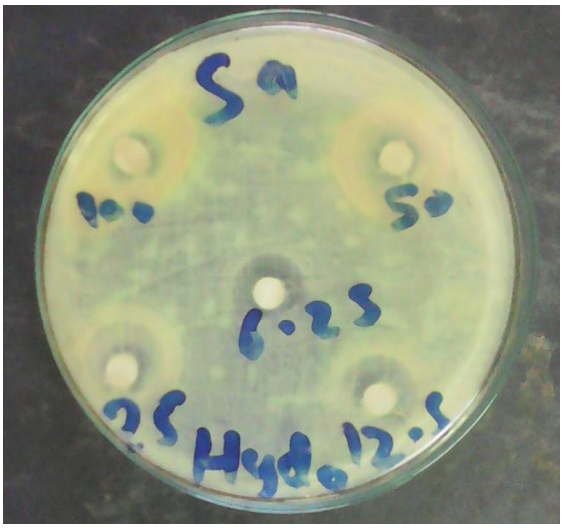
Table (3.2) Antibacterial activity for different concentrations of plant extract

	Antibacterial activity diameters (mm)			
Concentrations of extracts mg/ml	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus	Bacillus subtilis
100	20	22	20	21
50	19	21	18	20
25	18	20	17	15
12.5	17	19	15	_
6.25	16	13	13	_

Fig 3.1: antibacterial susceptibility Disc diffusion

Escherichia coli

Bacillus subtilis



Pseudomonas aeruginosa

Staphylococcus aureus

3.1.4 XRF analysis

Table (3.3) chemical composition of sample extract

composition	%
Organic content	96.610
Na as Na2O	0.260
Mg as MgO	0.042
Al as Al2O3	0.114
Si as SiO2	0.260
P as P2O5	0.289
S as SO3	0.461
K as K2O	1.067
Ca as CaO	0.033
Rb as Rb2O	0.001
Br as Br^-	0.005
Fe as Fe2O3	0.017
Ni as NiO	0.002
Cu as CuO	0.004
Zn as ZnO	0.006
Cl as Cl^-	0.830

3.1.5 FTIR analysis

Fig 3.2: showed the FTIR spectrum of Hydnora

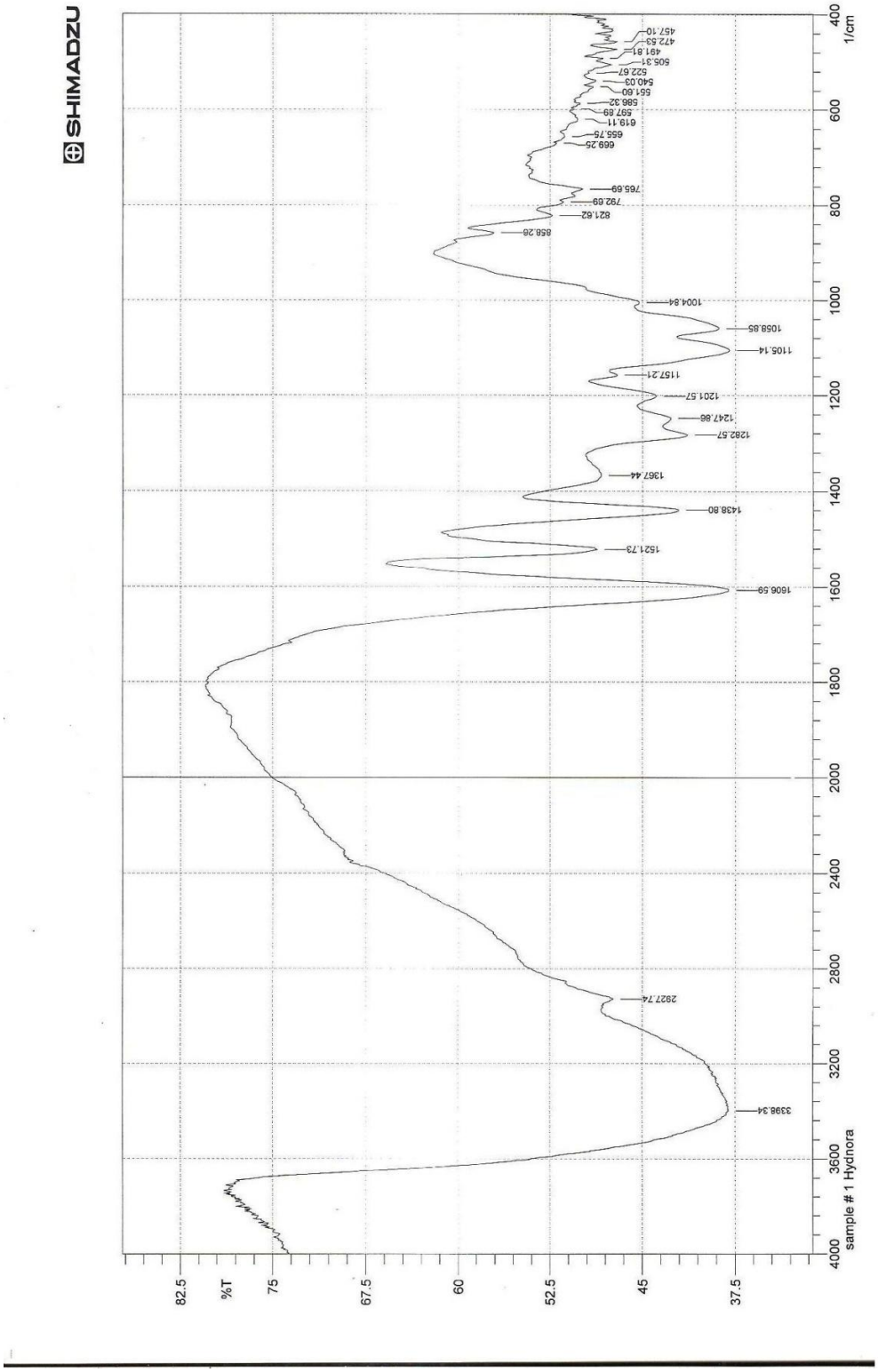


Fig 3.2 The FTIR spectrum of Hydnora

Table (3.4) Hydnora absorption peaks (IR)

NO.	Peak(cm^{-1})	Functional group
1	3398.34	-O-H (stretch)
2	2927.74	-C-H (stretch, sp^3, aliphatic)
3	1606.59	C=C (stretch)
4	1521.73 and 1438.8	Benzene ring (stretch)
5	1282.57	-O-H (bending)
6	1058.85	-C-O (stretch)
7	765.69	Benzene ring (stretch) (di substitution)

3.2 Discussions

3.2.1 Ash content

Ash content was found = 3.81% and this means that the inorganic components are few in the plant sample.

3.2.2: Antimicrobial test

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. In the present study, the extracts obtained from hydnora abyssinica show strong activity against most of the tested bacterial strains.

The antibacterial activities of plant extracts have been linked to the presence of some bioactive compounds or secondary metabolites. These secondary metabolites provide protection to the plants themselves against bacterial, fungal and viral infections. The extract that was tested against the 4 pathogenic bacteria, the bacteria with zones of inhibition varying from 13 to 22 mm (Table 2).

The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active. (Sana Mukhtar and Ifra Ghori 2012).

3.2.3 XRF analysis

Table 3 showed the elements present in extract of Hydnora abyssinica. Potassium (K) is found to show the highest concentration among the other elements.

3.2.4 FTIR analysis

In case of the extract of Hydnora absorption peak is appeared at 3398.34 cm-1 and the bond is -O-H type and these bonds appearance is broad. The second peak is appeared at wave number 2927.74 cm-1 and the bond is C-H sp^3.and the peak 1606.59 cm-1 and the bond is C=C type and these bonds appearance is strong. The absorption peaks at 1521.73 and 1438.8 cm-1 could be assigned to the functional group of benzene ring. And absorption peak at 1282.57 cm-1 and the

functional group is –O-H bend. And absorption peak at 1058.85 cm-1 and the functional group is C-O in alcohol or phenol and absorption peak at 765.69 cm-1 and the functional group is di substitute aromatic ring. And this is mean that compound is may be phenolic compound.

Conclusion

The composition of hydnora abyssinica root extract sample, from chemical and antibacterial site of view are determined.

Recommendations

It is concluded from the present study that these plant can be used to produce new therapeutics and it can be used to develop new antimicrobials.

Further research is required to investigate the bioactive molecules of hydnora abyssinica.

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