WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 116-124 Research Article ISSN 2278 – 4357

PHYTOCHEMICAL PROPERTIES AND PROXIMATE CONSTITUENT OF ASS HAY (DONKEY SKIN)

Iloputaife E. J.,*[1] Enweani I. B.[2] and Mbah-Omeje K. N.[3]

[1,3]Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

[2]Department of Medical Laboratory Science, Nnamdi Azikiwe University, Akwa, Anambra State Nigeria.

Article Received on
02 Feb. 2019,

Revised on 23 Feb. 2019,
Accepted on 16 March 2019

DOI: 10.20959/wjpps20194-13473

***Corresponding Author**
Iloputaife E. J.
Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

ABSTRACT

The present study investigates the phytochemical properties and proximate constituent of ass hay.The ass hay extracts were subjected to quantitativeandqualitative phytochemical screening and proximate analysis. The qualitative phytochemical screening of the aqueous, ethanolic and methanolic extracts of ass hay revealed the presence of saponins, glycosides, alkaloids and steroids. While tannin, flavoniods, terperniods, phenol and resin were absent. Quantitative phytochemical screening of the different samples of ass hay revealed varying quantities of the secondary metabolites in the different samples; glycoside was highest (13.54%) in oven dried sample and lowest (8.80%) in the sun dried sample, Alkaloids was highest (7.11%) in fresh sample and lowest (1.49%) in oven dried sample, Saponins was highest (5.49%) in fresh sample and least (1.07 %) in sun dried sample. Proximate analysis of Ass Hay indicated that Ass Hay contains a relatively appreciable quantity of the proximate components which includes lipids, fiber, protein, moisture and carbohydrate though at varied quantities with oven dried samples showing the highest ash and crude fiber contents. The presence of phytochemicals and appreciable quantity of proximate components from this study has shown the antimicrobial potential of ass hay and at such the need to further exploit donkey ass hay in order to maximize its potential.

KEYWORD: Aqueous, ethanolic and methanolic.

INTRODUCTION

Over the years, there has been significant search for components that possess antimicrobial activities. This search has gained increasing importance in recent times, because of the growing worldwide concern about the dire increase in the rate of infection by antibiotics-resistance microorganism and as a result of situations where some common and less expensive antimicrobial agentsare beginning to lose their effectiveness (Shittu*et al*., 2007). Researchers are in search of natural medications with fewer side effects.

Donkeys have in recent times been speculated to possess antimicrobial properties, even in view of the various uses of the donkey in Nigeria. The so much interest in donkey skin and milk by the Chinese merchants which is tied directly to the movement en masse of the animal from the Northern part of Nigeria, to others parts is because the importance of donkey in Chinese traditional medicine, particularly in anti-ageing solutions popular with women. (Vincent *et al*., 2016).

Among the most important set of Persian beliefs about the magical properties of the donkey is that connected with folk medicine, which may reflect an association with Christ. Applying three drops of liquid extracted from donkey dung, either mixed with other ingredients or alone,has been reported to stop most nosebleeds and heal most wounds. Smoke from burning ass's dung has been employed in fumigation because it is considered medicinally effective. Burning donkey's hooves to fumigate the genitals of women in labor was once thought to help in difficult childbirths (Omidsalar and Omidsalar, 1995).

In Iran, inflammatory oral ulcers such as aphthous ulcers and other inflammatory conditions such as infections of the middle and external ear have been treated with no side effects using anbarnesa smoke derived from burning female donkey's dung collected in spring. Sameanbarnesa smoke has been used in treatment of vaginal infections and decreasing the duration of menstrual period and stanch bleeding (Hassan *et al*., 2015).

Donkey milk has been shown to contains a number of antimicrobial factors, especially milk proteins, milk protein play a major role in milk protection. The enzyme lysozyme in donkey milk possess bactericidal property, a unique characteristicwhich makes it different from other mammal milks. The antimicrobial activity of donkey milk has been reported against *Salmonella choleraesuis* and *Shigelladysenteriae* (Zhang*et al*., 2008). The flesh of donkey

has also been considered a very effective antidote to poisons. As a result of these discoveries, the need for critical evaluation of the efficacy of the donkey skin is therefore imperative.

The aim of this study was to determine the phytochemical properties and proximate constituent of ass hay and predict its antimicrobial properties. This could support its use in place of synthetic drugs.

MATERIALS AND METHODS

Collection and Preparation of the Sample

Ass hay samples were purchased from Eke Abakaliki market, Ebonyi state. Samples were grouped into three batches, fresh, sundried and oven dried. Sundried and Oven dried samples were ground using a mechanical grinding machine; and sieved with a 0.5µm wire mesh. Fresh ass hay sample was chopped into tiny pieces with a sterilized knife and immersed in solvents (water, ethanol and methanol) for extraction. Extracts from the 3 batches of the ass hay were obtained using aqueous, ethanol and methanol solvents. Aseptic measures were observed.

Qualitative Phytochemical Screening Tests for Ass Hay

Various extracts from the three batches of ass hay were subjected to qualitative phytochemical screening using standard phytochemical methods as described by Harborne (1998) to determine the presence of: alkaloids, saponins, terpenoids, steriods, anthraquinones, coumarins, flavonoids, tannins, carbohydrate, reducing sugars, starch, resin, protein, amino acids, glycosides, phenolic content and flavonoid content.

Quantitative Phytochemical Analysis of Ass Hay

Various extracts from the three batches of ass hay were subjected to qualitative phytochemical screening using standard phytochemical methods as described by Harborne (1998) to determine the followings: alkaliods, saponins and glycosides contents.

Proximate Analysis

Proximate analysis was carried out using standard methods as described by AOAC (1990).

Moisture Content Estimation

One (1) gram of each of the various samples from the three batches of the Ass Hay were weighed into a crucible of known weight before oven drying in an oven for 2hr at 105˚c after

which they were left to cool in a desiccator, taking note of the new weight and then oven dried for another 30mins to get a constant weight.

% moisture content loss = W1 – W2/ WT ×100/1

Ash Content Estimation

One (1) gram of each of the various samples from the three batches of the ass hay were weighed into a crucible of known weight and ash using a muffle furnace at 600˚C for 3 hours after which they were left to cool and weighed.

Ash content W2 – W1

% Ash content W2 – (W1/WT) ×(100/1)

3.5.3 Crude Fiber Content Estimation

Two (2) gram of the various samples from the three batches of the Ass Hay were placed into a 500ml conical flask and added 200mls of 1.25% (v/v) H_2SO_4, heated for 30mins on a water bath (this is called acid treatment) after which they were filtered and the residues washed with hot water to neutralize the acid and re-soaked with 200ml of 1.25% sodium hydroxide for another 30mins (this is called base treatment). The solutions were filtered in a noted weight of filter paper and dried in an oven and weighed again. The contents were transferred into a weighed crucible and ashed using a muffle furnace, after ashing, they were cooled and weighed again.

Weight of fiber = weight of residue – weight of ash

% crude fiber = fiber weight/sample weight × 100/weigh

Protein Content Estimation (Kjeldahl Method)

A 0.5gof samples from the three batches of the Ass Hay were weighed each into a Kjeldahl flask and added 10grams of sodium sulfate, 1gram of copper sulfate and 20ml conc. sulfuric acid. The mixture was subjected to heat with Bunsen burner till the solution digests completely (changes to bluish green), then it was cooled and added 200ml of distilled water. Four (4) % boric acid in distilled water was prepared and added 2 drops of screen methyl red indicator, placed at the receiving end of the distillation apparatus. From the digested sample, 20ml of it was pipetted into a distillation flask and 10mls of 40% sodium hydroxide and few pieces of zinc metal were added and heated to distillate ammonia containing protein into the solution of 5mls of the 4% boric acid (note it color will change from pink to light or orange. There after the distillate were titrated with 0.1M HCL to pink colour).

TV × 0.001412 × 6.25 × DF × 100 / Weight of sample.

Fat Content Estimation (Soxhlet Extraction)

Fat extraction was accomplished using soxhlet extraction method by weighing 5gextracts from the three batches of the Ass Hay, wrapped very well in a filter paper and put in a soxhlet extractor while applying a heating mantle below a conical flask with n-hexane inside the flask which will help in extracting the oil from the sample. The system of extraction was recycled for 8-9 times to achieve maximum yield of oil, after which a dried beaker was weigh and the oil- n-hexane transferred into it and evaporated to get the crude oil yield then it was cooled and weigh again.

$$\text{Weight of oil} = W2\text{-}W1$$

$$\% \text{ lipids} = W2 - W1/\text{Weight of sample} \times 100/1.$$

Statistical Analysis

The tests were carried out in triplicate and values for the inhibition zone diameter reported as mean ± standard deviation, also the data obtained were subjected to one-way ANOVA.

RESULTS

Qualitative Phytochemical Screening of Ass Hay

Qualitative phytochemical screening of the aqueous, ethanolic and methanolic extracts of ass hay revealed the presence of saponins, glycosides, alkaloids and steroids while tannin, flavoniods, terperniods, phenol and resin were absent (Table 1).

Quantitative Phytochemical Analysis of Ass Hay

Quantitative phytochemical analysis of the different samples of ass hayas revealed that glycoside varied in quantity in which it was highest in oven dried sample and least in sun dried sample, alkaloids were found highest in fresh sample and least in oven dried sample and saponins highest in fresh sample ad least in sundried samples (Table 2).

Proximate Analysis

The oven dried samples showed the highest ash (3.4572 %) and crude fiber (14.1825 %) contents. The sun dried sample showed the highest lipid (12.848%) and carbohydrate (69.1155%) contents. While the fresh samples showed the highest moisture (16.2279%) and protein (27.0331%) contents (Table 3).

Table 1: Qualitative Phytochemical Screening Tests Results for Ass Hay.

Phytochemicals	Oven dried Aqueous	Oven dried ethanol	Oven dried Methanol	Sun dried Aqueous	Sun dried Ethanol	Sun dried methanol	Fresh water	Fresh ethanol	Fresh methanol	Remarks
Saponins	+	+	+	+	+	+	+	+	+	Present
Tannins	–	–	–	–	–	–	–	–	–	Absent
Flavonoids	–	–	–	–	–	–	–	–	–	Absent
Glycosides	+	+	+	+	+	+	+	+	+	Present
Alkaloids	+	+	+	+	+	+	+	+	+	Present
Steroids	+	+	+	+	+	+	+	+	+	Present
Terpeniods	–	–	–	–	–	–	–	–	–	Absent
Phenol	–	–	–	–	–	–	–	–	–	Absent
Resin	–	–	–	–	–	–	–	–	–	Absent

Table 2: Quantitative Analysis of Ass Hays.

Parameters	Sample	Average Concentration (%)
Glycosides	Oven dried	13.54
	Sun dried	8.80
	Fresh	21.53
Alkaloids	Oven dried	1.49
	Sun dried	2.66
	Fresh	7.11
Saponnin	Oven dried	4.72
	Sun dried	1.07
	Fresh	5.49

Table 3. Proximate Analysis of Ass Hay.

Sample	Moisture (%)	Ash (%)	Crude Fiber (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
Oven Dried	1.3335	3.4572	14.18255	10.92285	10.4483	59.6556
Sun Dried	0.9518	2.0089	10.6983	4.3775	12.848	69.1155
Fresh	16.2279	2.9377	3.36085	27.0331	3.39015	47.0503

Chi Square Value = 6.000.

P value = 0.1999, significant at 5% level with 10 degree of freedom.

DISCUSSION

Secondary metabolites (like saponins, glycosides, alkaloids and steroids) in the aqueous, ethanolic and methanolic ass hay extracts in synergy with other phytochemicals are responsible for the antimicrobial activities. Esam*et al*. (2017) attributed that the antibacterial activity of the methanol and aqueous extracts of *Typhadomingensis*Pers. to the presence of secondary metabolites like alkaloids, tannin, steroids, phenol, saponins, flavonoids compound, although the samples of Esam were not from ass hay. Alexeyena*et al*. (2009) reported the antimicrobial property of *Typhaangustifolia* Linn. to be as a result of the presence of saponins, alkaloids, steroids, phenols and tannins, although their samples were not collected from Ass Hay.

The low concentration of saponins in the oven dried (4.72 %), sundried (5.49 %) and fresh (1.07 %) batches of the Ass Hay could mean that ass haywill have minimal antimicrobial activities against Fungi, although the antimicrobial mechanism of phytochemicals varies; saponins have been reported to possess strong antifungal activities (Aboh*et al.*, 2014). This strong antifungal activity has been linked to the formation of complexes with sterol in fungal plasma membrane leading to death by destruction of cellular semi-permeabile membrane. George (2002) in his study reported that the interaction of saponins with cell membrane

sterols to be the major mechanism of the anti-fungal activities of saponins. Saponins had also been reported to show high antimicrobial activities against wide range of pathogens (Shibatas, 2001).

The presence of numerous phytochemical constituents in ass hay confersantibacterialproperty on it and hence prophylactic importance. Adegoke and Adebayo (2009) adduced the inhibitory activities of *Corchorusolitorius* leaf extracts on bacterial isolates to the presence of numerous phytochemical constituents, which can equally be of prophylacticimportance. Glycosides are endowed with diuretic biological activities, being already used in therapy as pharmacological tools. Among the reported biological effects, they present antitumor, anticholinergic, diuretic and anti-inflammatory properties Nonetheless, there were reports of toxic effects of glycosides to humans (El-shazly and Wink, 2014). The presence of alkaloids, steroids and saponins in the ass hay confers antimicrobial potency on it.

Carbohydrate and protein may be a conglomerate of bioactive sugars, glyco-proteins or proteins which give medicinal potency against certain diseases. These findings collaborate the study of Edeoga*et al.* (2006) which highlighted carbohydrate and protein to be a conglomerate of bioactive sugars, glyco-proteins or proteins which have medicinal potency. Ash content (Oven dried = 3.4572 %) (Sundried = 2.0089 %) and (Fresh = 2.9377) is an indication of mineral content. It implies that Ass Hay contained an appreciable amount of minerals due to its ash component.

REFERENCES

1. Aboh, M. I., Olayinka, B. O., Adeshina, G. O. and Oladosu, P. Antifungal Activities of Phyto Compounds from *Mitracarpusvillosus* (Sw.) DC from Abuja, Nigeria. *Journal of Microbiology Research,* 2014; 4(2): 86-91.
2. Adegoke, A. A. and Adebayo–Tayo, B. C., Phytochemical Composition and antimicrobial effects of Corchorusolitorius leaf extracts on four bacterial isolates. *Journal of Medicinal Plant Research,* 2009; 3(3): 77–80.
3. Alexeyena, V., Usha, G., Suja, A., and BGT, P., Sathianarayanan, Jose Asha. Phytochemical screening and antimicrobial investigation of Typhaangustifolia Linn. *Int J Chem Sci*, 2009; 7(3): 1905-1910.
4. AOAC. Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemists, Washington Dc., USA., 1990; 200-210.

5. Edeoga HO, Omosun G, Uche LC Chemical composition of *Hyptissauveolens*and *Ocimumgratissium* hybrids from Nigeria. *African Journal of Biotech*nology, 2006; 5(10): 892-895.
6. El-Shazly, A. and Wink, M. Structures, distribution, and biological properties of pyrrolizidine alkaloids of the Boraginaceae. *Diversity*, 2014; 6: 188–282.
7. Esam J. A., Yasamine, J. A., Khaled. K. A. and Hind, M. M. Physicochemical, phytochemical profiling and Biological activities of leaves extract of Bardy (Typhadomingensis Pers.) from Al-Chibayish marshes in southern Iraq. AASRC-Arf Joint International Academic Congress On Politics, Engineering, Sociology, Information, Health and Medical, Education and Communication, October, 2017; 25-26.
8. http://proceedings.sriweb.org/akn/http://arab.kmshare.net/.
9. George, F., Zohar, K., Harinder, P., Makkar, S. and Klaus B. The biological action of saponins in animal systems: a review, *Brit. J. of Nut.*, 2002; 88: 587–605.
10. Harborne, J. B. Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition, Chapman and Hall Ltd, London, 1998; 21-72.
11. Hassan, S.M., Byrd, J.A., Cartwright, A.L. and Bailey, C.A. Hemolytic and antimicrobial activities differ among saponin-rich extracts from guar, quillaja, yucca, and soybean. *Appl. Biochem. Biotechnol*, 2010; 162: 1008–1017.
12. Omidsalar, M. and Omidsalar, T. Donkey InPerisian tradition and folk belief. Encyclopedia Iranica, 1995; 5: 495–498.
13. Shibata S. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. *J. Korean Med. Sci.*, 2001; 16: 28–37.
14. Shittu, L. A. J., Bankole, M. A. Ahmed, T. and Akinsanya, M. A. Antimicrobial and antifungal activities of the crude extract of sesame radiation against some common pathogenic Microorganisms. *Journal of Ethno Pharmacology*, 2007; 21: 161-167.
15. Vincent, A. Y., Yusha'u, A., Ibrahim, K., Aliyu, M. Hamagam, and Onuchukwu, B. Chinese are going gaga for Nigerian donkeys. In Daily Trust, 2016.
16. Zhang, X., Zhao, L., Jiang, L., Dong, M. and Ren, F. The antimicrobial activity of donkey milk and its microflora changes during storage. *Food Control*, 2008; 19: 1191–1195.