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PRELIMINARY INVESTIGATION ON BIOACTIVE COMPONENTS AND PROXIMATE ANALYSIS OF METHANOL LEAVE EXTRACT OF MITRAGYNA INERMIS

YAKUBU S.; HYCENTH I. ABDU; & REJOICE A. J.

Department of Science Laboratory Technology, Federal Polytechnic, Mubi Adamawa State, Nigeria

ABSTRACT

Mitragyna inermis is a medicinal plant that has been used in traditional medicine for the treatment of diabetes, ulcer, pile, dysentery, and borne pain among the Hausa/ Fulani extractions of the Northern Nigeria. It has also shown efficacy on malaria, boils, arthritis, epilepsy, rheumatism, stomach pain etc. this research is undertaken to investigate the

INTRODUCTION

Man and plant had been together right from the time of creation. This suggest man's dependence on plants for shelter, food and medicine. Plants through natural processes synthesizes variety of chemical compounds which are potent in nature and physiologically active (keritorich, 2005). The phytochemicals which are commonly found in plants are flavonoids, alkaloids, saponins, tannins etc. (Ismail et al., 2005) and Namukobia et al., 2011.) These bioactive compounds are the major players in traditional medicine and development of pharmaceutical drugs. The significance of plant derived compounds in the field of medicine has triggered a continuous research on medicinal plants, where thousands of newer compounds are reported yearly. These phytochemicals are referred to as secondary metabolites, they are usually subjected to pharmacological testing, modification and derivatization for development

of new drugs. (kayser et al., 2000; Veena et al., 2014). Large number of these phytochemicals have been shown to be safe and effective with less adverse effect as antimicrobials, antioxidant, antidiarrheal, anticancer and wound healing (Yakubu et al., 2010).

Apart from the use of plants in the field of medicine, it is also utilized as a good source of food for both man and animal (Akpabio et al., 2012). Reports have revealed that the effectiveness of plant parts, used as food or medicine depends on nutrients and the bioactive compounds present.

The Genus *Mitragyna* belong to *Rubiaceae* family and is found in swampy area in the tropical and sub-tropical region of Asia and Africa. It is a medicinal plant commonly used in traditional medicine for the treatment of different kinds of diseases with a well-known efficacy across Africa, (Wakirwa et al., 2013; Konkon et al 2008). A concoction prepared from the bark of *Mitragyna* has been found to be effective against Syphilis, epilepsy, fever, dysentery, high blood pressure. The roots have been shown to be active against constipation and

bioactive and nutritional components of the leaves of the plant. Bioactive components of the plant was investigated using Thin Layer Chromatography (TLC) while the proximate analysis was done according to AOAC methods. The TLC method showed the presence of ten phytochemicals based on the number of spots on the chromatogram. Tannin was spotted using 10% ferric chloride solution. The proximate composition showed moisture content to be 48%, total ash 9.0%, crude fibre 25%, crude protein 4.9%, and carbohydrate as 4.6%. The presence of bioactive components justifies the usage of the plant in traditional medicine. It is equally an indication that the plant when subjected to pharmaceutical processes, can be used in the development of drugs. The results further suggest the relevance of the plant in nutraceuticals

Keywords: *Mitragyna inermis, proximate composition, bioactive compounds, Thin Layer Chromatography*

leprosy, the leaves treats jaundice, weakness, fatigue and as child birth stimulant etc. (Konkon, 2008; Wakirwa et al., 2013)

This study is aimed at investigating bioactive components and at the same time evaluating the nutritional potential of the leaves of *Mitrgyna inermis.*

MATERIALS AND METHODS

Chemicals and reagents

The chemicals and reagents used were of analytical grade purchased from reliable dealer (Sunlab Chemicals, Jos, plateau state. They includes; n. hexane, toluene, acetone, sodium sulphate , cuper sulphate , sulphoric acid, formic acid, ferric chloride, TLC paper, iodine crystals .

Plant collection and authentication

The leaves of *mitragyna inerms* was collected from Funtuwa Katsina state and authenticated by the Federal College of Forestry Jos, Plateau state.

Preparation of plant extract

The leaves were washed with a clean water and allowed to dry under room temperature. It was then grinded into powder using electric blender.

Thin Layer Chromatography

Thin Layer chromatography was performed using standard methods (Harbone 1998) the commercial standard TLC was used with particle size range. Small portion of the extract was (2mg/ml) was dissolved in acetone. Small spot of the solution containing the sample was applied on the plate 1 cm from the bottom marked by a line.

Spotting and development

The spotted plate was well dried before placing in a chromatographic tank containing 6:6:1 acetone, toluene and formic acid as solvent system. The upward movement of the spot was then monitored. When the solvent reaches the peak of the plate it was removed, marked and dried.

Iodine tank

After the pots were separated, the chromatogram was removed and the spots circled with pencil. The spots were identified by observation using physical eyes and iodine vapor

Identification of Bioactive Components

Ferric solution was prepared by dissolving 0.1g in 10ml of distilled water. The solution was applied on the chromatogram for the identification of tannins.

Proximate Analysis

The proximate analysis of the sample for moisture, crude fiber, fat, and carbohydrate were carried out using AOAC, (2002) method. While the crude protein and the ash content were carried out using method described by Ega *et al.*, (1990)

RESULTS AND DISCUSSION

Results

Table 1: Thin Layer chromatography of *Mitragyna inermis* leave extract

spot	*Sf*	*DMS*	R_f*-Value*	*color*
1	8.5	8.2	0.96	light green
2	8.5	6.8	0.8	yellow
3	8.5	6.5	0.76	yellow
4	8.5	5.9	0.69	yellow
5	8.5	5.4	0.63	brown
6	8.5	4.7	0.55	d/green
7	8.5	2.8	0.32	brown
8	8.5	2.2	0.25	brown
9	8.5	1.7	0.20	brown
10	8.5	1.1	0.12	brown

Table 2: Nutritional composition of Mitragyna interims

Serial no.	Constituents	weight (%)
1	Moisture	48
2	Ash content	9

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3	Crudes fiber	25
4	Crude protein	4.9
5	Carbohydrate	4.6

Discussion

Tin Layer Chromatography

The results of the Tin Layer Chromatography (TLC) of *Mitragyna inermIs* leave extract are presented in table 1. From the table of results, there are ten (10) different phytochemicals present in the leave extract as designated by the number of spots on the chromatogram. The constituents were distinct, considering the rf values and the color of spots. Eight of the spots were visible to the eye, while two (8 and 10) were only visible in iodine vapor. Spot one (1) has the rf value of 0.96, yellow in color. Spots 2,3 and 4 were yellow in color with rf values of 0.80, 0.76 and 0.69 respectively. Spot 5 has the rf value of 0.63 brown in color while spot 6, has rf value of 0.55, dark green in color. Spots 7,8,9 and 10 have the rf values of 0.32, 0.25, 0.20 and 0.12 respectively, all bearing the same color (brown).

A ferric chloride solution (10%) applied on the chromatogram reacted with spots 7,8,9 and 10, giving a blue-black color which is a reaction peculiar to tannins. Hence the results depicts 7,8,9 and 10 as tannins.

Tannins are toxic to fungi, yeast and bacterial, their presence in the medicinal plants therefore, suggests the ability of the plant to play key role as antifungal, antidiarrheal, antioxidant and antihemorrhaoid agent (Kumanye, 1992)

Tannins are used in wine as astringent agent that modifies its taste as well as in the development of pharmaceutical drugs. (Loi MC et al., 2002)

Proximate Analysis.

The proximate composition of the leave extract of *Mitragyna inermis* is presented in table 2. The results indicated that the leave contain moisture (48%) ash (09%), crude fibre (25%), crude protein (4.9%) and carbohydrate (4,6%). The moisture content is higher than the reports earlier presented for some legumes; *tetracarpidium Conophorum* (43%) (Ogbonna et al.,2013), *Zinger offinale* roots (15.02%), (Shina et al., (2010).

Further reports by Gbadamasi et al., (2014) showed *Carlpolobia intea* roots, and *Microdenus perberula* have 8.9% and 8.75 respectively. However, the results of proximate analysis of the leaves of *C. ambrosioides* (89.40), O. *gratissimum* (80.80), *V. amgygaldalina* (79.20), *M. lucida* (70.20), *P. nigrescens* (70.01), *M. indica* (60.40) and *B. buonopozense* roots (53) as conducted by Biodum et al.,(2017) and (Basey et al., 2016) indicated higher percentage of moisture content. Moisture content of a plant is highly significant, considering the role it plays in determining the activities of enzymes and co-enzymes, needed for plant metabolism. Therefore, the conducive environment needed by organs for effective functioning is dependent on the moisture content of the plant (Iheanacho and Udebuani, 2009; Iroko et al., 2014; Basey et al., 2016). On the other hand, high moisture content, indicates susceptibility to spoilage if not well stored. (Omoregie and Osagie, 2011)

From table 1 above, the ash content of the sample is low (09%) when compared to *C. ambrosoides, V. amygaldalina, O. gratissimum, M. lucida, P. nigrascens and M. indica* leaves which were 17%, 17.2%, 14.30%, 12.25, 12.20 and 12.04 respectively (Abiudum et al., 2017). The results however are similar to that of *B. buanopozense* (9.59%) reported by Basey et al., (2016) and higher than that of *A. sativum* reported by Hussain et al., (2009). The ash content is determined after burning all organic components of the plant. The ash is the inorganic constituents that indicates the amount of mineral element present in plant sample. The results therefore indicates low amount of mineral elements in the plant.

The percentage crude fiber in *M. inermis* as presented in table 1 above, was 25%. The value depicts appreciable amount, which is significantly higher compare to *C. ambrosoides, V. amygaldalina, O. gratissimum, M. lucida, P. nigrascens and M. indica* leaves which were 13.04, 15.06, 22.02, 19.01, 22.05,22.01 respectively (Abiudum et al., 2017). Furthermore, findings documented by Ogbonna et al., (2013) for *tetracarpidium conophorum* roots (7.43), *L. cuponiodes* roots (15.50), *Ocoba spinose* roots (14.25), *Microdesmis puberula* roots (14.00) were lower. However, *Zingiber officinale* roots(49), *Datura innoxia* roots (31) and *P. thonningii* (35,03), have significantly higher percentage of crude fiber than *M. inermis.* (Ene-Obong et al., 1992); (Shina et al., 2010) and (Ayuba et al.,

2010) the value of crude fiber found in *M. inermis* could be considered among many plants as a good source of crude fibre, which when consumed adequately lowers the serum cholesterol level, heart disease, hypertension, constipation, diabetes and breast cancer.(Ishida et al., 2000) and (Abiodun *et al* .,2017)

The percentage crude protein as presented in table 1, was 4.6. Which is relatively low, When compared with *V. amygaldalina* (18.22), *C. ambrosioides* (11.30), *P. nigrescens* (10.20) , *M. lucida* (10.20), *O. gratissimum* (10.05), *M. indica* (9.05) Abiodun et al., 2017). Similarly, *L. cuponiodes* roots (17.55), *Carpolobia lutea* (15.25) *Ocoba spinose* roots (17.05), *Microdesmis puberula* roots (16.85) (Gbadamosi *et al* ., 2014) are higher than *M. inermis.* The results reported for *Talinum triangulare* (5.09), *Zingiber officinale* (5.08) are similar, to *M, inermis*, while that for *Amaranthus hybridus* (4.80) and *Gnetum africanum* (3.15) are lower, (Asquith and Butter, 1986). Protein constitute a vital component of human diet required by the body for the replacement of dead tissues, production of energy and supply of adequate amount of needed amino acids (Igile *et al*., 2013) and (Shemishere et al., 2018) consumption of the leaves of *M. inermis* may not supply the protein required by the human body.

The results of proximate analysis for *M. inermis* leaves is shown in table 1. The leaves contain low level of carbohydrate (4.6) compare to *V. amygaldalina, M. lucida, O. gratissimum, C. ambrosioides, M. indica and P. nigrescens* which were 54.00, 51.66, 50.6, 43.76, 40.23 and 36.03 respectively. It is also low compared to *L. cuponiodes* roots (45.70), *Carpolobia lutea* (52.10), *Microdesmis puberula* roots (58.85), and *Ocoba spinose* roots (51.25) as documented by Gbadamosi et al., (2014). The carbohydrate content of milk apple, white apple and water apple were, 8.65%, 12.68% and 8.49% which are relatively low and cannot be considered as good source of carbohydrate as reported by Adeleke and Abiodum (2010) in line with this reports, *M. inermis* is not a good source of energy.

Conclusion

This study revealed that *M. inermis* is an important source of bioactive components. Particularly the presence of tannin suggests the ability of

the plant to play key role as antifungal, antidiarrheal, antioxidant and anti-hemorrhoid agent. With the global quest for phyto-medicines, it can be incorporated as a potential source for the development of novel drugs. The proximate analysis unveiled the crude fibre potential of the plant which is present in an appreciable quantity. Which can be harnessed for possible application in food formulations owing to its numerous advantages such as lowering of cholesterol, heart disease, hypertension, diabetes, etc. It is also essential to identify all the spots and determine their microbial activities, while on the other hand toxicity studies need to be carried out to determine its safety levels.

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