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Analysis of Aqueous and Ethanolic Leaf Extracts of *Momordica charantia* (Bitter Gourd) using High Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared (FTIR) Spectroscopy

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Abstract

The study of analysis of aqueous and ethanol leaf extracts of M. charantia was carried out using High Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared spectroscopy (FTIR). The qualitative phytochemical analysis of aqueous and ethanol extracts showed the presence of alkaloid, flavonoid, glycoside, saponins, terpenoids, steroids, and tannins. The result of quantitative phytochemical/phytonutrient of the aqueous and ethanol extracts revealed the highest concentration of Ascorbic acid (31276.43±0.015), thiamine (713.27±0.020), Alpha-tocopherol (714.67±0.011), and beta-carotene (68.35±0.012) µg /100 g in ethanol than aqueous leaves extracts. However, cardiac glycosides in both the aqueous and ethanolic extracts show the value 42.54±0.015 mg/100 g and 61.44±0.006 mg/100 g respectively. Whereas total sugar, 0.1865±0.001 %, and water soluble protein 0.0415±0.001 % and reducing sugar 0.0745±0.001 % has the lowest value both in aqueous and ethanol extracts. The result of FTIR peak value and functional group of aqueous extract have strong adsorption peaks at 3302.88 cm^{-1} (sharp) 2936.74 cm^{-1} (broad), 1556.11 cm^{-1}; (Sharp) 1410.94 cm^{-1} (Small), which correspond to the presence of hydroxyl (OH), alkane (C-H) Carbonyl and aromatic rings functional groups respectively. Ethanol extract showed broad peak value at 3309.77 cm^{-1} of OH groups, sharp and strong peak 2927.84 cm^{-1} of alkane, small and weak peaks of alkenes 1714.43 cm^{-1} and 1591.29 cm^{-1} ethyl acetate. Therefore, the aqueous and ethanolic crude extracts are rich source of mineral which could be beneficial to human beings, while taking the extract for more than a week are strongly inhibited many pathogenic bacteria and fungi strains.

Keywords: *M. charantia;* Qualitative phytochemical analysis; Quantitative phytonutrient; aqueous leaf extract; Ethanolic leaf extract

Introduction

Plants play a prominent role in maintenance of human health and have been used as medicine since ancient times. According to the estimation of World Health Organization (WHO) (1995), plant extracts are used as medicines in traditional therapies by 80% of the World's population (Baker *et al.*, 1995) and more than 30% of the plant species have been used for medicinal purposes (Joy *et al.*, 1998). The use of plants as sources of drugs, vegetables and foods cannot be underestimated. Virtually all plants are medicinal hence they serve as raw materials for synthetic drugs (Sofowora, 1993). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Antony *et al., 2*013) Therefore, the analysis of these bioactive constituents would help in determining various biological activities of plants. These bioactive substances which can be present in all plant parts include terpenoids, steroids, saponins, tannins, flavonoids, alkaloids (Sofowora, 1993). The medicinal plants of Africa accounts for nearly two third of the total plants species used in modern system of medicine and in rural areas as tea, extracts. Herbal drugs are widely prescribed, even when their biological ingredients are not known, due to their effectiveness, fewer side effects and low cost (Kumar *et al.*, 2009; Ajayi *et al.*, 2011). The rational design of novel drugs from traditional

medicine obtained from plant offers new prospects in modern health care (Manjamalai *et al.,* 2010).

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend them against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 of such compounds have been isolated so far; a number estimated to be less than 10% of the total (Tapsell, 2006). Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. The conventional medicine is more than the herbal medicine in terms of their standards and purity (Lai & Roy, 2004).

The use of plants as medicines predates written human history (Fabricant & Farnsworth, 2001). Ethno botany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethno medical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, opium etc. (Fabricant & Farnsworth, 2001).The use of herbs to treat diseases is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicines for some aspects of primary health care (Edgar *et al.*, 2002). Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available.

Momordica charantia is a species of *Momordica* belonging to the *Cucurbitaceae* family with the common name, bitter mole, or bitter gourd, (English). In Adamawa State it is used as food as well as medicine. It is not formally cultivated as a commercial crop anywhere in the world (Makgakga; 2004). It is normally cooked with pounded groundnut (peanut butter) and beans to serve as dish and to improve the flavour. This research is limited to leaves of *M. charantia* grown in Mubi North Adamawa State.

The Aim of the Study is to evaluate the phytochemical contents of the aqueous and ethanolic extracts of *Momordica charantia* then purification and characterization of the active functional groups with available spectroscopic techniques.

Materials and Methods

Sample Collection and Authentication

Fully matured dark green leaves of *M. charantia* were collected in and around the vicinity of Mubi North Local Government Area, Adamawa State in the month of July, 2018. Plant species was authenticated in the Department of Botany Adamawa State University, Mubi- Adamawa State.

Sample Preparation and Extraction

The plant leaves were thoroughly washed with tap water and rinsed with distilled water to remove dust and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were shade dried and pounded/pulverised to powdered form using pestle and mortar.

Extraction of Sample with Ethanol and Aqueous

The powdered leaves (100 g) were weighed and soaked in 350 mL of ethanol in a volumetric flask. The flask with instantaneous shaken was corked and left to stand for 48 hrs at room temperature. After 48 hrs, the mixture was filtered using Whatman Filter Paper No. 1, the filtrate was concentrated using Rotary evaporator and water bath at 38 ^{0}C to dryness (Evans and Trease, 1996).

Phytochemical Screening

The leaves extracts were subjected to preliminary phytochemical screening using methods described by (Evans and Trease, 1996 and AOAC 2000). Various qualitative and quantitative chemical tests were conducted for detection of alkaloids, terpenoids,

phenols, steroids, flavonoids, tannins, carbohydrates, glycosides and saponins in aqueous and ethanol extracts of the leaves.

Table 1: Quantitative Phytonutrients composition of *M. charantia* extracts in micro gram per 100grams

Phytonutrients µg/100g	Aqueous leaves Extract	Ethanol leaves Extract
Ascorbic acid	27857.14±0.02	31276.44±0.02
Retinol	4.25±0.01	4.77±0.01
Alpha tocopherol	642.14±0.03	714.66±0.01
Beta carotene	62.76±0.01	68.35±0.01
Psi- carotene	9.26±0.01	11.59±0.02
Thiamine	642.55±0.01	713.26±0.02
Nicotinamide	2.44±0.01	3.45±0.02
Terpenoids	1.06±0.02	1.77±0.01

The values are in terms of mean ±SD of results done in triplicate

Table 2: Quantitative Phytochemical composition of *M. charantia* extracts in milligram per100grams.

Phytochemicals mg/100g	Aqueous leaves Extract	Ethanol leaves Extract
Steroids	2.35±0.01	2.90±0.02
Phlobatannins	2.66±0.02	3.10±0.04
Tannins	1.34±0.17	1.94±0.01
Cardiac glycoside	42.55±0.02	61.44±0.01
Total phenols	0.84±0.01	1.45±0.01

The values are in terms of mean ±SD of results done in triplicate

Table 3: Quantitative Phytochemical/Phytonutrient composition of *M. charantia* extracts in percentage.

Phytochemicals/Phytonutrients (%)	Aqueous leaves Extract	Ethanol leaves Extract
Alkaloids	12.64±0.01	15.43±0.01
Flavonoids	7.36±0.01	8.07±0.01
Saponins	2.16±0.06	2.86±0.01
Total protein	1.46±0.06	1.45±0.02
Water soluble protein	0.04±0.01	0.09±0.01
Total sugars	0.19±0.01	0.28±0.01
Reducing sugars	0.08±0.01	0.13±0.01

The values are in terms of mean ±SD of results done in triplicate

Therefore, all the phytochemicals/phytonutrients analyzed in aqueous extracts shows lower values than the ethanol extracts (table's 1- 3).

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transform infrared spectroscopy (FTIR) spectra analysis was utilized to identify the functional group of the active ingredients on the basic of peak values in the vicinity of infrared radiation. The result of FTIR peak values and functional groups of *M. charantia* aqueous extract are present in (Table 4). Spectrum shows strong adsorption peaks at 3302.88 cm^{-1} 80.26% Transmittance (Broad), 2936.74cm^{-1}; 92.26% T Sharp and strong peak 1556.11cm^{-1}; 73.62% T (Sharp) 1410.94 cm^{-1}; 76.98% T (Small), which correspond to the presence of hydroxyl (OH) Alkane (C-H) and aromatic rings functional groups respectively.

Table 4: Fourier transform infrared spectroscopy FTIR spectral peak values and functional groups obtained for the aqueous leaf extract of *M. charantia*

Extracts	Peaks value cm-^{1}	% Transmittance	Functional Groups
Aqueous	3302.88	80.26	OH- Group
	2936.74	92.26	C-H Stretching
	1556.11	73.62	C=O Group
	1410.94	76.98	C-H bending in CH_2
	1047.63	81.43	C-O Group

M. charantia ethanol extract (Table 5) showed broad peak value (3309.77cm^{-1}; 82.15% T) of OH groups, sharp and strong peak (2927.84cm^{-1} ; 81.89% T) of alkane, small and weak peaks of alkenes (1714.43cm^{-1} ; 83.39% T) and (1591.29cm^{-1} ; 72.47% T) ethyl acetate respectively.

Table 5: Fourier transform infrared spectroscopy FTIR spectral peak values and functional groups obtained for the ethanol leaf extract of *M. charantia*

Extracts	Peaks value cm-^{1}	% Transmittance	Functional Groups
Ethanol	3309.77	82.15	OH- Group
	2927.84	81.89	C-H Stretching
	1714.43	83.39	C=O Carbonyl group
	1519.29	72.47	C=O Group
	1375.08	72.41	C-H Bending
	1041.32	55.64	C-O Group

Discussion

The qualitative phytochemical analysis of aqueous and ethanolic leaf extracts of *Momordica charantia* revealed the presence of alkaloid, flavonoid, glycoside, saponins, steroid, tannins, terpenoids and phenols. Phytochemicals are plant secondary metabolite that have been reported to have many biological and therapeutic uses, so the presence of these phytochemicals is expected to make the plant a potential for many medicinal uses table 1 (Vishnu *et al.,* 2013 and Narender *et al.,* 2012).

Alkaloids are the most significant compounds that play a metabolic role in the living systems and are involved in the protective function in animals. Flavonoids are best known for their antioxidant and anti-inflammatory health benefits as well as the support of the cardiovascular and nervous systems (Alexopoulos *et al.,* 2008). Flavonoids have been used against the cancer causing tumors and then inhibit the promotion of growth and progression of tumors (Stevens *et al.,* 1992). Phenols, when mixed with the flavonoid compounds in plants are reported to show multiple activities like antioxidant, anti-carcinogenic, anti-inflammatory etc (Asha *et al.,* 2011). The leaf extracts of *Momordica charantia* was found to contain high amount of ascorbic acid and is comparable to the one found in orange and other fruits. Ascorbic acid scavenges free radical that triggers the inflammatory cascade such as in asthma, osteroarthritis, and rheumatoid arthritis. It also boosts the immune system (Zablotowicz *et al.,* 1996). The extract of *Momordica charantia* is therefore a good source of antioxidant. Retinol and β-carotene play a vital role in free radical scavenging activities and also vital role in bone growth and teeth remineralization (Gropper *et al.,* 2009).

Thiamine was found (Table 1) in both extracts which plays a vital role in maintaining central nervous system. It also increases glucose uptake and energy secretion with the help of nicotinamide through glycolysis, tricarboxylic acid cycle and electron transport chain. (Antony et al., 2013). Steroids were found to be 2.35±0.012 and 2.90±0.015 mg/100 g in aqueous and ethanolic extracts respectively. Steroids are precursor for cholesterol synthesis which is the cell membrane components that serve as signal molecules with active steroid hormone-receptor (Chung *et al.,* 1998). Tannins are water-soluble polyphenols that are present in many plants. They have been reported to be responsible for decreases in

feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value (Chung *et al.,* 1998). Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure. Their utility results from an increased cardiac output by increasing the force of contraction. They also delay depolarization thus decreasing heart rate *(Wang, et al., 2008).* Vitamin E (α- tocopherol) is a fat soluble vitamin and potent antioxidant that is believed to be important in protecting cells from oxidative stress, regulating immune function, maintaining endothelial cell integrity and balancing normal coagulation (O'Neil, 2013). Nicotinamide is an important compound functioning as a component of the coenzyme nicotinamide adenine dinucleotide (NAD). Nicotinamide is the active form of Vitamin B_3 and a component of the coenzyme nicotinamide adenine dinucleotide (NAD). Niacinamide acts as a chemo- and radio-sensitizing agent by enhancing tumor blood flow, thereby reducing tumor hypoxia, (O'Neil, 2013).

The FTIR spectroscopy is an excellent technique for the investigation of biological structures due to its sensitivity and ability to give valuable information about the functional groups, which might have diagnostic value for biological systems. The value of infrared spectral analysis comes from the fact that the modes of vibrations of each group are very sensitive to changes in chemical structures, conformation and environment. The results of the present study show that FTIR spectroscopy is a very informative technique to differentiate aqueous and ethanol leaves extracts at the molecular level. The results of FTIR peak values and functional groups were represented in (Tables 4 and 5) for different compounds. The solvent had its respective functional groups like alkane, alcohol, ethers. Hence, the crude extract subjected to FTIR analyses was used for the identification of chemical constituents present in *M charantia.* In addition, FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition (Komal *et al.,* 2011).

The use of herbs in the management of ailment has been a regular practice in Africa with considerable therapeutic success. *M. charantia* is a plant highly praised for its nutritional and therapeutic benefits. The leaves of *M. charantia* were extracted with N-hexane, ethyl acetate and methanol, ethanol and quite a good yield was obtained from all the solvents (Orafidiya *et al.*, 2000).

Conclusion

The aqueous and ethanolic crude extracts of *M. charantia* are found to contain high phytonutrients. *M. charantia* leaves are a rich source of mineral which could be beneficial to human beings, while taking the extract for more than a week are strongly inhibited many pathogenic bacteria and fungi strains.. The finding of the study revealed that *M. charantia* are used as traditional herbal medicine, containing a number of useful phytochemicals and providing data base line information for further primary health care system.

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